Bioindication capacity of fish parasites for the assessment of water quality in the Danube River

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``… as an ecosystem matures, parasitism naturally tends to evolve into mutualism; parasites that fail to make that transition end up destroying their host and consequently themselves. Human society must make the same transition … from exploitation of the natural environment to harmonious interaction with it. The danger of destroying our host, the planet earth, was new because until recently neither the size of the human population nor the extent of humans' technological manipulation of the environment had been great enough to affect regional and global balances.``

Eugene Odum

(Craige, 2001)

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Glossary

Ammonium EDTA – ammonium Ethylendiaminetetraacetic acid. EDTA salts are used as a chelating agent for metal ions.

BCF – Bioconcentration factor. Calculated for each analyzed element according to Sures *et al.* (1999*a*) as a ratio between the metal concentration in the parasite and the host tissue $C_{[P.laevis]}$ / $C_{[host\ tissue]}$ as well as between the parasite and the concentration in the water $C_{[P.laevis]}$ / $C_{[water]}$. It represents an arithmetical approach for expressing the accumulation capacity of fish acanthocephalans.

bp – base pairs. Pair of nucleotides (bases) which are complementary bounded. In the molecular biology, the number of base pairs is used as an important measure for the size of a particular gene or for the entire genome.

DNA – Deoxyribonucleic acid. The most important feature of the DNA molecule is to store the genetic information, which is important for functioning and development of the living organisms.

DORM-3 – Fish protein certified reference material for trace metals. The reference material is used for control and verification of the entire analytical procedure, which was performed in the thesis.

ICPDR – International Commission for the Protection of the Danube River. The Commission works to ensure the sustainable and equitable use of waters and freshwater resources in the Danube River Basin. The work of the ICPDR is based on the Danube River Protection Convention, the major legal instrument for cooperation and transboundary water management in the Danube River Basin (ICPDR, 1998).

ICP-MS – Inductively Coupled Plasma Mass Spectrometry. This is a methodology for measuring of numerous metals, which includes inductively coupled plasma for ionization and mass spectrometer for detecting the ions. ICP-MS is a rapid and highly sensitive technique in the field of analytical chemistry.

ITS – Internal Transcribed Spacer is a region of ribosomal DNA (see rDNA). Comparison of the sequence of ITS regions is a commonly used approach in taxonomical studies due to their high variation between close related species.

JDS – Joint Danube Survey sampling sites. The abbreviation in combination with the numbers (e.g. 13, 16, 26 and 32) was used in chapter 4 to represent the localities from which the fish samples during JDS2 were sampled.

JDS1 – First Joint Danube Survey. A scientific expedition along Danube River carried out in

2001. It delivered various analyses of the water quality and ecological status of the Danube River and some tributaries (JDS, 2001).

JDS2 – Second Joint Danube Survey. The JDS2 is known as the world's biggest river research expedition. It was performed in 2007 and delivered profoundly information about water quality and pollution in the Danube River and some of its tributaries (JDS, 2007).

PCBs – Polychlorinated biphenyls. They represent a group of toxic organic compounds used mainly in the industry as dielectric fluids for transformers and capacitors. Their molecule is formed by up to ten chlorine atoms attached on biphenyl (two benzene rings).

PCR – Polymerase Chain Reaction. This is a common technique in the field of molecular biology, applied to amplify/generate from one or few pieces of DNA thousands/millions of copies of a particular DNA sequence.

rDNA – ribosomal DNA. It represents those sequences of the DNA, which include the genes of the ribosomal Ribonucleic acid.

SPM – Suspended Particulate Matter. It represents the suspended sediment fraction in the water phase. SMP regulates the transport of all types of water pollutants in dissolved and particulate phases.

TNMN – TransNational Monitoring Network, in short ``TNMN`` was established to support the implementation of the Danube River Protection Convention in the field of monitoring and assessment. It was formally launched by the ICPDR in 1996. The main objective of the TNMN is to provide a structured and well-balanced overall view of pollution and long-term trends in water quality and pollution loads in the major rivers in the Danube River Basin (TNMN, 1996).

List of used element abriviations – arsenic (As), bismuth (Bi), cadmium (Cd), colbalt, (Co), copper (Cu), iron (Fe), mercury (Hg) manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), tin (Sn), titanium (Ti), vanadium (V), zinc (Zn).

Background

In recent years aquatic ecosystems suffer from a permanent increase of pollution caused by the industrialization and urbanization. Simultaneously, the humans continue to extend their knowledge regarding the problems emerging after and try to study in detail every component of the ecosystem in order to understand the consequences of such external stress. In general, ecosystems are complex systems consisting of a number of mutual interacting components. Observed independently, each part (component) of a given ecosystem represents a piece of a puzzle. Combining each of the puzzle pieces should deliver an entire picture of the ecosystem condition. The size of the puzzle varies according to the size and complexity of the ecosystem. Therefore, for obtaining precise information over its general condition, we need to explore as much as possible available parts. At this point the ecologists set the concept for ecosystem health, which is a measure of how every piece of the puzzle match the entire puzzle and how are they balanced, if we continue thinking abstractly. Costanza and Mageau (1999) defined the ecosystem health as a '...comprehensive, multiscale dynamic, hierarchical measure of system resilience, organization and vigor.' The concept comprises the system's ability to keep its structure (organization) and function (vigor) over time with regard to external stress (resilience). In simple words a healthy ecosystem is one which comprises a balance between system components, stability, diversity and complexity, absence of disease and last but not least homeostasis. All these aspects are summarized in the term ``ecosystem sustainability``, which is actually the overall performance of the system resulted from the interaction and behavior of its components (Costanza and Mageau, 1999).

In the field of ecological monitoring, researchers are trying to study as many parts of a given ecosystem as possible in order to detect external stress factors, which mostly occurring in the form of contamination. The chemical (all external substances, which naturally do not belong to the system) or physical (thermal, noise, radioactive etc.) contamination itself can induce changes in the ecosystems functionality and structure, which on the other hand affects its overall performance. Therefore, ecological monitoring is mostly aimed at studying the changes that could be assessed after exploring in detail the balance between the system components. Following the history of hydrobiological monitoring, at the beginning (until the middle of the 19th century) water quality assessment was based only on some chemical or physical parameters of water bodies. Kolenati (1848) and Cohn (1853) for the first time discovered and described that some organisms are showing a relation to the water quality (summarized by Bock and Scheubel, 1979). At the beginning of the 20th century Kolkwitz and

Marsson (1902, 1908, 1909) found a close relationship between water organisms and pollution after studying the biological and chemical processes of self-purification running in lotic ecosystems (mostly in River Rhine). A methodology (the Saprobic System) for hydrobiological monitoring based on animal communities labeled as bioindicators was developed and established for first time. Furthermore, the water quality assessment implemented more and more components over time, after analyzing their relationship with pollution. This implies macroinvertebrate communities, macrophytes, algae, fungi, fish, even ciliats have been studied from a bioindicator perspective. Worth noticing is that all these groups (components) have a basic common characteristic – they are an inseparable part of aquatic ecosystems. But there are still some components less investigated. One of them could be the group of fish parasites. The presumption, that aquatic parasites have no relation to the environment conditions prevailed for quite a while, arguing with the parasite's specific biology. Fish parasites were always underrated by field ecologists in aquatic monitoring, because they lacked in most of the cases ``direct`` connection with the ambient water medium. They were observed mostly from the perspective of water born diseases or some breakout infection events in the fish populations, without searching the reasons which in term laid mostly on the disturbed environment conditions, respectively pollution. In the last couple of decades, after gathering more detailed information concerning these aspects, many studies showed that fish parasite communities also react to alterations in conditions. Furthermore these alterations resemble those of free living organisms. The first evidence was delivered by impact surveys on some ectoparasitic species of fish, particularly on monogenean trematodes. They are common fish parasites occurring on gills and skin, therefore they are in permanent contact with the surrounding environment. By observing monogeneans presence or absence and diversity characteristics of their communities, it is possible to obtain valuable information about the alternation in environment factors (summarized by Sures, 2001). Thus, their close relation to eutrophication processes was demonstrated (Koskivaara, 1992; Valtonen *et al.* 1997), as well as to other pollution sources like effluents from the industry (e.g. pulp and paper mills) (Siddall *et al.* 1997). This relation was mostly expressed by reduced species richness and unequal distribution of abundances (summarized by Sures, 2001). However, this parasite group exhibit some features similar to free living organisms, which are also in permanent contact with the surrounding environment.

However, endoparasitic assemblages, although ``embodied`` in the host, may also have a relationship to pollution. Thus, the first step to achieve an understanding for the interaction between parasites and environmental factors is to get an overview on the parasite

transmission. Despite the high variety and complexity in transmission, the larger part of the endoparasites exhibit stages affected by the environment conditions. The direct effect is normally expressed by lethal reactions of the free living larval stages (e.g. Metacercaria) or adults, whereas the indirect impact is addressed on the intermediate or final host – the pollution could drive the suitable intermediate and final hosts to extinction (Sures, 2008*a*). It can also affect the host physiology and thus the infected host as well as the parasites may suffer more from environmental exposure. In both cases the pollution leads to changes in the diversity and richness of parasite communities and thus parasites can be used as **effect indicators**. For that reason the parasite communities are more frequently analyzed in respect to pollution in the last decades. In summary, the effect indicators deliver information about the ecosystem health and integrity through changes in diversity and structure of their communities (Sures, 2001). However, should an ecosystem rich in parasites be considered as healthy? In the review paper published by Hudson *et al.* (2006) the position of parasites on the ecosystem level and their important regulatory role for the entire biodiversity and production was clearly defined. Therefore, the parasite's diversity and richness is as important as those of the other ecosystem components like producers and consumers, which always have been in the focus of ecologists.

In addition to the ecological aspects of bioindication, fish parasites could be also an appropriate tool for detecting and quantifying some toxic substances in aquatic habitats. Recently, the intensive research on their application as sentinels showed that they are even more advantageous than the already established organism (Sures *et al.* 1997*a*, 1999*b*). Due to their enormous accumulation capacity, parasites such as acanthocephalans can concentrate toxic chemicals (e.g. heavy metals) even though the ambient concentrations are far below the detection limits – this is advantageous especially in some less polluted habitats like the Antarctic (Sures and Reimann, 2003) or for substances in very low concentration ranges, like precious metals (Sures *et al.* 2005). In general, **accumulation indicators** are organisms, which are able to accumulate substances (in the most cases toxic) from the surrounding environment within their bodies and thus deliver information about the bioavailability of the given substance and its environment contents. Various experimental and field studies demonstrated and proved parasite's sentinel features, whereas the most promising group was found to be the group of fish acanthocephalans. They are widely spread intestine parasites of fish, characterized with a relative short life cycle (Kennedy, 2006). The experiments on heavy metal uptake mechanism showed that the accumulation process start immediately after the infection of the definitive host, whereas the uptake occurs through gills over the circulatory

system and entero-hepatic route of the fish (Sures and Siddall, 1999). Thus, this considerably fast mechanism of accumulation leads to achievement of steady state concentrations of the particular metal in parasite after only 4-5 weeks after the first exposure (Sures, 1996), which makes the acanthocephalans a very sensitive and quick instrument for the detection of metal pollution.

Regardless, further investigations of fish parasites in respect to their bioindication features are needed, in order to be applied in the aquatic monitoring. There are still uncertainties regarding the ideal sentinel organism (summarized by Sures, 2003; **Table I**); however, if the fish acanthocephalans are taken as metal indicators these issues should be overcome. The table listed below shows in summarized form the information which is available or is still missing:

Table I. List of criteria characterizing the ideal sentinel organisms according to Martin and Coughtrey (1982), Philips and Segar (1986), Phillips and Rainbow (1993) - summarized by Sures (2003) for acanthocephalans.

Criteria	Acanthocephala
Rapid equilibrium whith the source	Yes
A linear relationship with source over the range of ambient concentrations	Yes
The relationship between the tissue and source concentrations should be the same at all sites studied	1
Abundant species from which large numbers can be taken without altering the age structure or having some other significant effect on population	Yes
Easily identified	Yes
Large body of knowledge about the species' physiology, including the effects of age, size, season and reproduction activity on the assimilation of the pollutant	No
Large body- to provide abundant tissue for analysis	Yes
Sedentary or with a well defined home range	Yes
Uptake is from a well defined pollution source	Yes
Easily aged and long lived - allowing integration of the pollutant over long periods	1

1 More information required

According to **Table I** it seems that acanthocephalans fulfill almost all necessary criteria regarding their application as sentinels. The lack of knowledge concerns mostly some uninvestigated aspects of their biology such as effects of the age and the size of the

acanthocephalans as well as the effects of the seasonality and reproduction which might induce oscillations in the accumulation process. As summarized by Sures (2003), the only disadvantage, which the acanthocephalans probably exhibit, is that they are hard to be aged and are not long living animals. However, the short life spawn can be put to an advantage, as acanthocephalans could possibly deliver a more precise chronological view on metal pollution than other organisms, postulated their life spawn is restricted to an exact timeframe (e.g. year). Consequently, it could be able to date accurately the pollution sources and events, when they occur and subsequently manage them. Therefore, some further investigation concerning the live duration of the parasites is required.

Even if ecologists are able to fill those knowledge lacks, a logical question appears: Do we need to implement actually new bioindicators in our hydrobiological praxis?

The need of parasites as accessory bioindicators can be also seen as gathering additional knowledge over their ecological state, and thus we will improve our view on the overall condition on ecosystem level. They are an additional piece of the puzzle, which we need to collect if we want to obtain a more detailed picture of ecosystem's homeostasis and integrity. Therefore, it can be concluded that the water quality could be assessed more precisely by using accessorily the fish parasites as bioindicators, especially in large and complex lotic systems like Danube River, where the conventional hydrobiological methods exhibit some intricacies. The implementation of fish as bioindicator during the second monitoring expedition in 2007 (Joint Danube Survey) was an example that the hydrobiologists need to extend their monitoring spectrum to achieve and enhance the desired information about the ecological state of Danube River. The fish parasites, like fishes, are an inseparable part of aquatic ecosystems, therefore they should also be taken into account by hydrobiological monitoring.

Scope of the thesis

The objective of the current thesis is to expand the scientific basis concerning employing fish parasites as bioindicators. Therefore, a field investigation was carried out as a monitoring survey over four years from summer 2004 to summer 2007. It covers some faunistical and ecology aspects of fish parasites in relation to environmental conditions (**Chapter 1**). Furthermore, the thesis intends to cover the lack of knowledge regarding the application of fish acanthocephalans as sentinels for metal pollution (**Chapter 2** and **Chapter 3**). Additionally, it delivers a detailed heavy metal monitoring over the investigation period (**Chapter 4**).

Chapter 1: The endohelminth fauna of barbel (*Barbus barbus*) correlates with water quality of the Danube River in Bulgaria

This chapter gives an overview on the endohelminth fauna of the barbel in the lower Danube for the period summer 2004 to summer 2007. The composition and diversity of the parasite communities were studied in seasonal manner at different sampling sites in Bulgaria in order to express the capacity of fish parasites as effect indicators. The possible variation in the composition and diversity of their communities was expected to be related to the local environmental conditions.

Chapter 2: Is metal accumulation in *Pomphorhynchus laevis* dependent on parasite sex or infrapopulation size?

The chapter covers some uninvestigated aspects regarding the application of fish acanthocephalans as accumulation indicators.

Two questions are in the main focus of this chapter:

Is the metal accumulation by *P. laevis* dependent on the parasite`s sex?

And: Does the size of the infrapopulation influence the metal accumulation in the parasite?

Chapter 3: Seasonal differences of metal accumulation in *Pomphorhynchus laevis* and its definitive host *Barbus barbus*

This chapter presents the effects of seasonality of *P. laevis* development on metal accumulation in the host-parasite system. Furthermore, according to the obtained data was designed a model, which represents the metal uptake process in natural conditions.

Chapter 4: Application of the acanthocephalan *Pomphorhynchus laevis* from its host barbel (*Barbus barbus*) as metal indicator in the Danube River

This chapter delivers a metal monitoring study conducted with the suggested barbel – *P. laevis* system. The data was supported with background chemical data delivered by the International Commission for the Protection of the Danube River (ICPDR) in order to express the bioindication capacity of fish acanthocephalan regarding heavy metals and arsenic.

1 The endohelminth fauna of barbel (*Barbus barbus*) correlates with water quality of the Danube River in Bulgaria

1.1 Introduction

In recent years, fish parasites attain increasing interest from an environmental point of view (Sures, 2006, 2008*a*). Many studies demonstrate the close relation between parasitism and ecological conditions in a given environment and describe how parasites can be used to enlarge knowledge on ecosystem function and integrity (Hudson *et al.* 2006; Lafferty *et al.* 2008). Pollution with toxic substances such as metals or polychlorinated biphenyls (PCBs) as well as an enrichment of nutrients (eutrophication) may affect the occurrence and physiology of parasites. The effects of toxic pollutants and eutrophication on parasites can be direct (e.g. by reduction of the number of free living stages or intermediate host) or indirect (e.g. host immunosuppression) depending on the pollution type and parasite life cycle (Sures, 2008*a*). Various studies demonstrate for example that euthrophication reduces the diversity of heteroxenous parasites, whereas parasites with direct life cycle (monoxenous) are less affected. The latter are often ectoparasites which are in direct contact to the surrounding water and are thus adapted to changes in environmental conditions (Valtonen *et al.* 1997; MacKenzie, 1999; Perez-del Olmo *et al.* 2007). Concerning toxic pollutants it emerges that certain substances such as metals or PCBs cause immunosuppression in the fish host and thus may increase parasitism by a reduced host defence (Hoole, 1997). The resulting numerical changes (increase or decrease of abundance and intensity) of aquatic parasites leading to changes in structure and diversity of parasite communities as a response to different forms of pollution may be used for bio-indication purposes (MacKenzie *et al.* 1995). Accordingly, the occurrence and diversity of parasites stand as a measure of ecosystem health even if the underlying functional chains are often unknown.

In order to use fish parasites as pollution indicators, the fish host must be widely distributed and easy to be sampled (Kennedy, 1997). Therefore, the present investigation was focused on barbel (*Barbus barbus*) and its parasite communities at different sampling sites along three lower reaches of the Danube River. The barbel is the second largest native cyprinid fish species in Europe, being wide spread in major European river systems. Although many studies on the parasite fauna of *B. barbus* have been published from selected localities of the Danube basin, data from east Europe and especially from the Balkan Peninsula and the Danube delta is scarce. Only few studies on parasites of barbel in the Danube River in Bulgaria (Kakacheva-Avramova, 1962, 1977, 1983; Margaritov, 1959, 1966; Nedeva *et al.*

2003) and in Romania (Roman, 1955) exist, whereas most information on barbel parasites is delivered from Central Europe (Michalovič, 1954; Moravec and Scholz, 1991; Moravec *et al*. 1997; Laimgruber *et al*. 2005). Until now, the complete endohelminth fauna of *B. barbus* reported for the Danube drainage system in Central Europe consists of 43 species with 22 trematodes, 9 cestodes, 7 nematodes and 5 acanthocephalans (Moravec *et al*. 1997). In contrast, the list of barbel endohelminths in the Bulgarian section of the Danube River (Kakcheva-Avramova, 1977) includes only 6 species, but there are a few unpublished studies, which describe up to 11 species.

The aim of the present chapter was to obtain a more complete picture of the endohelminth fauna of *B. barbus* and to study the composition and diversity of parasite communities with respect to the environmental conditions of the habitats. It is expected that the structure and diversity of parasite communities over consecutive years at sites that differ in their degree of eutrophication and in their concentration of toxic metals reflect the ecological conditions.

1.2 Materials and Methods

1.2.1 Sampling sites

The study was carried out in a seasonal manner (April, July and October) ranging from summer 2004 to summer 2007 at three different localities of the Bulgarian part the Danube River. The sampling sites (see **Figure 1.1**) were selected on the basis of different degrees of eutrophication and toxic pollutants, as the main objective of the current research was to check if parasite communities reflect the environmental conditions of their habitats. The first sampling site is located near Vidin (river kilometre 834), about 10-15 km away from the inflow of the river Timok (845 km), which is one of the biggest metal pollution sources downstream in the Danube. The second sampling site was selected near to town Kozloduy (685 km), approximately 160 river kilometres downstream from Vidin. The third site was on the border between Bulgaria and Romania near the town Silistra (375 km) which represents the last Bulgarian locality in eastward direction of the river. The sampling stretches covered approximately 5 river kilometres at each sampling site (**Figure 1.1**).

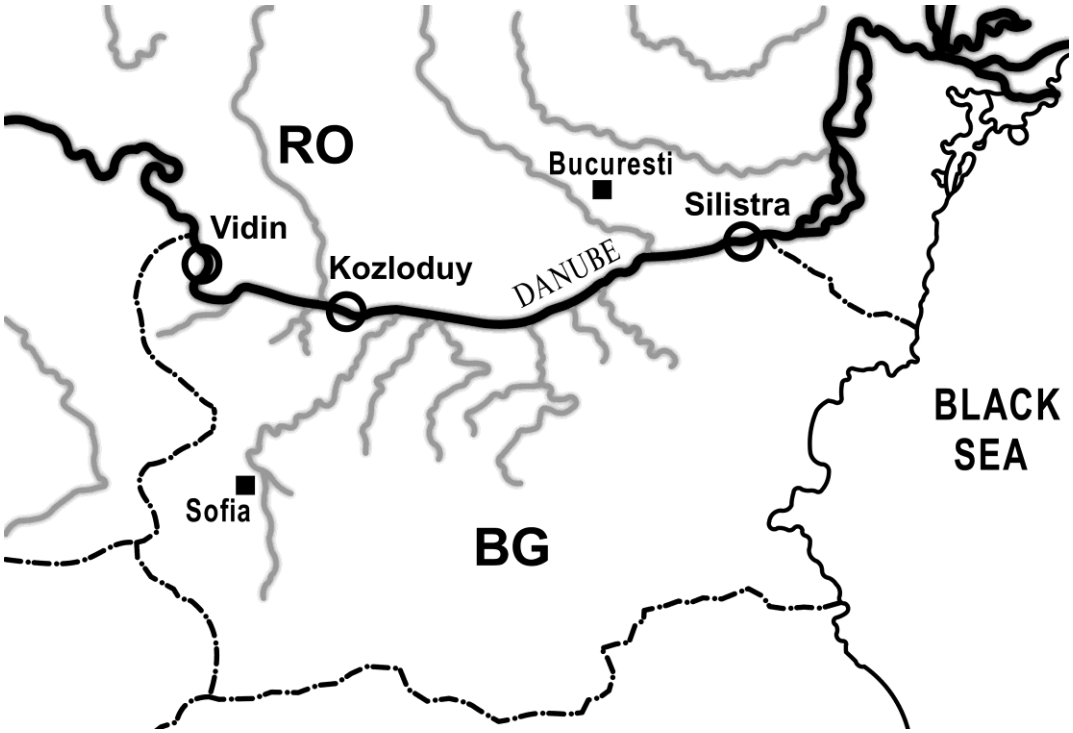

Figure 1.1. Location of the sampling sites along the Danube River in Bulgaria. BG- Bulgaria; RO- Romania.

1.2.2 Fish sampling

A total of 407 barbels were collected by fishermen, using drift nets. The number of individuals with a minimum total length of 20 cm varied between 10 and 35 fishes per sampling site and season (**Table 1.1**). During the whole sampling period a total of 165 fish were caught in Vidin and 193 in Kozloduy. Sampling continued for only two years in Silistra, where 49 barbels were sampled between 2006 and 2007. Additionally, spring sampling at all sites was performed only in the years 2006 and 2007. After catching, the fish were frozen at -15°C and transported to the laboratory, where total length (TL), standard length (SL), body weight (BW), sex and age for each fish was determined. The condition factor (K) was calculated as follows $K=100*BW*TL^{-3}$ (Schäperclaus, 1990). The fish were subsequently dissected and analysed for parasites using standard parasitological techniques. The skin, scales, fins, gills, eyes, gut, cavities and organs were examined using a stereomicroscope (magnification x8 to x50). Nematodes were fixed in 70% ethanol and mounted in glycerine for further identification whilst all other parasites could be indentified directly.

Table 1.1. Morphological parameters and characteristics of collected fish material.

Sampling sites	Sampling time	No. of fishes	Weight [g]		Total Length [cm]		Condition factor	
			mean ± SD	range	mean ± SD	range	mean ± SD	range
Vidin	Spring	48	702 (± 577)	108 - 3909	40.9 (± 8.3)	25.5 - 72	0.88 (± 0.10)	0.65 - 1.05
	Summer	58	836 (± 604)	207 - 2145	43.2 (± 10.4)	28.7 - 63.7	0.85 (± 0.08)	0.74 - 1.07
	Autumn	59	565 (± 486)	81 - 2390	38.5 (± 9.2)	23.4 - 67.2	0.83 (± 0.15)	0.30 - 1.28
	Total	165	649 (± 509)	81 - 3909	39.9 (± 8.8)	23.4 - 72	0.87 (± 0.14)	0.30 - 1.80
Kozloduy	Spring	37	599 (± 280)	120 - 1125	39.6 (± 6.3)	26 - 52.3	0.89 (± 0.10)	0.68 - 1.15
	Summer	86	587 (± 371)	125 - 2208	38.9 (± 7.6)	24.5 - 60.5	0.88 (± 0.09)	0.64 - 1.07
	Autumn	71	539 (± 370)	140 - 1785	38.5 (± 7.9)	26.2 - 56.9	0.86 (± 0.16)	0.38 - 1.49
	Total	193	573 (± 355)	120 - 2208	38.9 (± 7.5)	24.5 - 60.5	0.88 (± 0.13)	0.38 - 1.49
Silistra	Spring	10	801 (± 216)	400 - 1050	43.2 (± 5.2)	34.8 - 50	0.99 (± 0.15)	0.77 - 1.28
	Summer	27	948 (± 367)	410 - 1785	44.7 (± 5.9)	34.1 - 54.8	1.01 (± 0.13)	0.80 - 1.31
	Autumn	12	624 (± 168)	420 - 900	39.7 (± 4.4)	34.3 - 47	0.99 (± 0.12)	0.71 - 1.16
	Total	49	838 (± 327)	400 - 1785	43.2 (± 5.7)	34.1 - 54.8	1.00 (± 0.13)	0.71 - 1.31

1.2.3 Determination of helminth community structure and statistical treatment

Parasitological parameters used followed those suggested by Bush *et al.* (1997) - prevalence (P, %), intensity range (IR), abundance (A) and mean intensity (MI) of the infection. The following diversity indices were calculated to describe the richness and diversity of the parasite communities: Brilliouin index (H_B), Shannon-Wiener index (H_S), Shannon-Wiener evenness (E), Simpson's index (D) and Berger-Parker index (d) according to Magurran (1988) and Sures *et al.* (1999*c*).

Correlations between intensity and fish weight were checked using Spearman's rank correlation coefficient. A one-way ANOVA was employed to determine significant differences in the diversity characteristics of the intestinal infra-community and to compare the number of each parasite species between sampling sites. For estimating differences of fish condition factors between sampling sites, the Mann-Whitney *U*-test was applied.

1.2.4 Water quality

Water quality data for sites adjacent to our fish sampling sites (see **Table 1.2**) were obtained from the technical reports published by the Joint Danube Survey (ICPDR, 2002, 2008*a*,*c*) and annual reports and the database of TNMN (Trans National Monitoring Network, ICPDR, 2004, 2005, 2008*b*). These research programs and activities are initialised by the International Commission for Protection of the Danube River (ICPDR). The available data were used as a basis to interpret the composition and richness of helminth communities at the same localities.

Table 1.2. Data on selected aqueous nutrient and pollution parameters according to ICPDR (2008*b*) for upper and lower sites of the Bulgarian part of Danube River.

Parameters	Year	Vidin[1]	Kozloduy[2]	Silistra
Ammonium	2003	0.185	0.265	0.079
[mg/L]	2004	0.191	0.183	0.075
	2005	0.207	0.288	0.078
	2007 *	0.016	0	0
Nitrate	2003	1.203	0.661	1.119
[mg/L]	2004	1.446	0.977	1.435
	2005	1.41	0.829	1.574
	2007 *	1.45	1.44	1.56
Nitrite	2003	0.033	0.022	0.019
[mg/L]	2004	0.025	0.021	0.02
	2005	0.032	0.022	0.016
	2007 *	0.059	0.064	0.016
Orthophosphate	2003	0.054	0.053	0.064
[mg/L]	2004	0.116	0.061	0.071
	2005	0.12	0.068	0.059
	2007	0.069	0.043	0.041
Total phosphorus	2003	0.323	0.108	0.119
[mg/L]	2004	0.184	0.103	0.164
	2005	0.21	0.130	0.149
	2007 *	n/a	n/a	n/a
Cadmium	2003	1	1.000	1
[µg/L]	2004	1	1.167	1
	2005	1	1.825	1
	2007 *	n/a	n/a	n/a
Copper	2003	14.9	9.083	6
[µg/L]	2004	18.7	6.417	2.5
	2005	17.5	5.158	1
	2007 *	n/a	n/a	n/a
Lead	2003	1.8	2.333	2.8
[µg/L]	2004	2	2.583	1
	2005	1.8	2.767	1
	2007 *	n/a	n/a	n/a

[1]: Sampling site Novo Selo, 1 km away from Vidin
[2]: Sampling site Iskar–Baikal, 40 km away from Kozloduy
*: Data delivered by 2nd Joint Danube Survey- Onboard results (ICPDR 2008a)
n/a: Data not available

1.3 Results

1.3.1 Total parasite fauna

A total of 10 endohelminth parasites species was recovered, including 3 trematodes (*Dipostomum spathaceum* (metacercariae) in the eye lens, *Posthodiplostomum cuticola* (metacercariae) on the skin, *Metagonimus yokogawai* (metacercariae) on the scales), 3 acanthocephalans (*Pomphorhynchus laevis*, *Acanthocephalus anguillae*, *Leptorhynchoides plagicephalus* in the intestine) and 4 nematodes (*Rhabdochona hellichi*, *Pseudocapillaria tomentosa*, *Hysterothylacium* sp. (larvae) in the intestine and *Eustrongylides* sp. (larvae) in the body cavity) (**Table 1.3**). One acanthocephalan species (*L. plagicephalus*) and 2 nematodes (larvae of *Eustrongylides* sp. and *Hysterothylacium* sp.) were recorded for the first time for barbel. Only one fish from the sampling site Vidin was infected with a single adult male of *L. plagicephalus*, which is thus considered an accidental infection. Larvae of *Hysterothylacium* sp. were found in the gut of one barbel collected at the sampling site Kozloduy. *Eustrongylides* sp. occurred at all sampling sites during the entire period. This nematode together with the nematode *R. hellichi* was the second most widely distributed parasite species at the sampling site Vidin (P, 24.2 %; MI, 10.1). Also at the sampling sites Silistra and Kozloduy it occurred with high prevalence and intensity (Kozloduy P: 17.1%; MI: 9.1; Silistra P: 14.3%; MI: 2.1). The pattern of infection presents a clear correlation between fish size, prevalence and intensity of infection. The highest prevalence was found in barbels with a length between 40 to 60 cm. Infection intensity increased significantly (Spearman correlation, $p<0.05$) with body size (Vidin: $r = 0.32$; Kozloduy: $r = 0.39$; Silistra: $r = 0.34$).

Total species richness ranged between 9 worms for Vidin and Kozloduy and 7 for Silistra. The most abundant parasite was the acanthocephalan *P. laevis*. At the sampling site Vidin 100% of the fishes were infected with this acanthocephalan and the mean intensity was 124.6 worms per fish. Only two fishes from Kozlduy (P, 99%; MI, 84.3) and one from Silistra (P, 98%; MI, 117.7) were not infected with *P. laevis*. The second most frequent species at all sampling sites was *R. hellichi*. The number of *R. hellichi* individuals showed significant differences between Vidin and Kozloduy ($p = 0.029$, $F = 4.795$), and Vidin and Silistra ($p = 0.003$, $F = 8.78$), whereas no differences were detected between Kozloduy and Silistra.

Table 1.3. Prevalence, mean intensity and mean abundance of the parasites of barbel from three sampling sites along the Danube River in Bulgaria.

Parasite species	Samppling site	Prevalence P [%]	Mean Intensity MI (± SD)	Intensity range	Abundance
Rhabdochona hellichi	Vidin	24.2	15.9 (± 35.6)	1 - 207	3.9
	Kozloduy	47.7	34 (± 99)	1 - 759	16.2
	Silistra	46.9	72.9 (± 180.7)	1 - 761	34.2
Pseudocapillaria tomentosa	Vidin	4.8	1.4 (± 0.7)	1 – 3	0.07
	Kozloduy	4,1	2.3 (± 2.0)	1 – 7	0.09
	Silistra	10.2	2 (± 1.7)	1 - 5	0.2
Eustrongylides sp. larv.	Vidin	24.2	10.1 (± 20.5)	1 – 93	2.5
	Kozloduy	17.1	9.1 (± 14.1)	1 - 68	1.6
	Silistra	14.3	2.1 (± 1.9)	1 - 6	0.3
Hysterothylacium sp. larv.	Vidin	-	-	-	-
	Kozloduy	0.5	1	1	0.01
	Silistra	-	-	-	-
Pomphorhynchus laevis	Vidin	100	124.6 (± 122.5)	1 – 874	124.6
	Kozloduy	99	84.3 (± 77.7)	2 – 424	83.4
	Silistra	98	117.7 (± 107.5)	4 - 523	115.3
Acanthocephalus anguillae	Vidin	1.2	1	1	0.01
	Kozloduy	0.5	2 (± 2)	2	0.01
	Silistra	-	-	-	-
Leptorhynchoides plagicephalus	Vidin	0.6	1	1	0.006
	Kozloduy	-	-	-	-
	Silistra	-	-	-	-
Diplostomum spathaceum larv.	Vidin	7.3	-	-	-
	Kozloduy	8.8	-	-	-
	Silistra	6.1	-	-	-
Postodiplostomum cuticola larv.	Vidin	16.4	-	-	-
	Kozloduy	17.1	-	-	-
	Silistra	38.8	-	-	-
Metagonimus yokogawai larv.	Vidin	12.7	-	-	-
	Kozloduy	15.5	-	-	-
	Silistra	10.2	-	-	-

The trematodes were the third group in terms of prevalence. Metacercariae of *P. cuticola* were most frequently found, followed by *M. yokogawai* and *D. spathaceum* at all sampling sites. There are no data available concerning the intensity of infection, since only the presence of metacercariae was recorded. The nematode *P. tomentosa* was present in all Danube sites during the whole sampling period. Whilst the prevalence was similar (4.8% and 4.1%) at the sampling sites Vidin and Kozloduy, it was more than 2 times higher in Silistra.

1.3.2 Diversity of helminth communities

Diversity and dominance indices were calculated without considering trematodes as they were not counted individually. Diversity characteristics of the infra-community are presented in **Figure 1.2** and **Table 1.4** and **Table 1.5**. Most of the fish were infected with either one or two parasite species simultaneously (**Figure 1.2**). At Vidin more than 50% of all fish were infected with one species only, whereas at Silistra 10% of the barbels were co-infected with 3 species. A clear increase in average diversity in downstream direction is reflected by the Brillouin index, which showed the highest value at Silistra. Statistical analyses revealed significant differences for the Brilliouin index between Vidin and Kozloduy ($p = 0.005$, $F = 8.101$) and Vidin and Silistra ($p = 0.038$, $F = 4.375$), whereas no difference was found between the sampling sites Kozloduy und Silistra ($p = 0.853$, $F = 0.034$). Concerning seasonal differences highest infracommunity diversity was found in spring and autumn for two sites, only Kozloduy showed a higher Brillouin index in summer than in autumn.

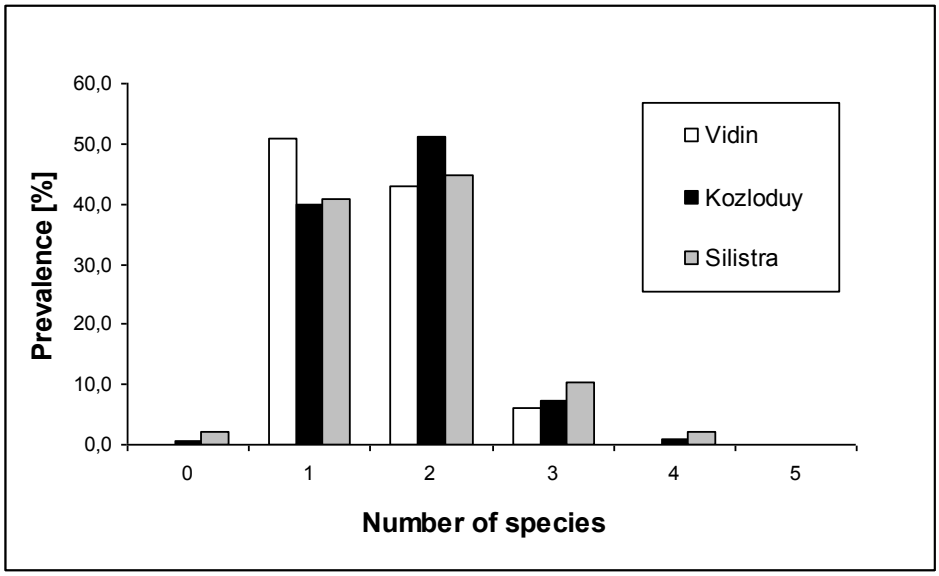

Figure 1.2. Prevalence of coexistent helminth species of barbel from three sampling sites of the Danube River.

Table 1.4. Average diversity characteristics of the infra community of helminths of barbel from the Danube River.

Sampling sites	Vidin	Kozloduy	Silistra
No. of barbels	165	193	49
Mean no. of helminth species per barbel ± SD	1.55 ± 0.61	1.68 ± 0.66	1.69 ± 0.77
Maximum no. of helminth species per barbel	3	4	4
Mean value of Brillouin's Index (H_B)± SD	0.10 ± 0.16	0.15 ± 0.19	0.16 ± 0.22
Maximum value of Brillouin's Index (H_B)	0.76	0.68	0.66

Table 1.5. Seasonal profile of the diversity characteristics of the infra community.

Sampling sites	Vidin			Kozloduy			Silistra		
	Spring	Summer	Autumn	Spring	Summer	Autumn	Spring	Summer	Autumn
No. of barbels	48	58	59	37	86	71	10	27	12
Mean no. of helminth species per barbel ± SD	1.71 ± 0.62	1.60 ± 0.65	1.37 ± 0.52	1.81 ± 0.66	1.67 ± 0.69	1.62 ± 0.62	1.40 ± 0.84	1.74 ± 0.81	1.59 ± 0.71
Maximum no. of helminth species per barbel	3	3	3	3	4	3	3	4	3
Mean value of Brillouin's Index (H_B) ± SD	0.14 ± 0.19	0.07 ± 0.12	0.09 ± 0.17	0.19 ± 0.23	0.15 ± 0.20	0.13 ± 0.17	0.16 ± 0.26	0.11 ± 0.18	0.23 ± 0.27
Maximum value of Brillouin's Index (H_B)	0.68	0.76	0.71	0.68	0.66	0.67	0.65	0.66	0.65

Similarly, component community diversity (**Table 1.6**) was also found to be higher downstream (Silistra) than upstream (Vidin). This tendency is also reflected by the Berger-Parker dominance index, for which highest values were found in Vidin and lowest in Silistra. Kozloduy showed medium values compared to the other sampling sites. Highest seasonal diversity was found in spring in Vidin and Kozloduy and in summer in Silistra (**Table 1.7**).

Table 1.6. Comparison of the average richness and diversity characteristics of the total component community of helminths of barbel.

Sampling sites	Vidin (n=165)	Kozloduy (n=193)	Silistra (n=49)
No. of helminth species	6	6	4
Shannon-Wiener Index (H_S)	0.23	0.52	0.56
Shannon-Wiener Evenness (E)	0.13	0.33	0.40
Simpson's Index (D)	1.10	1.42	1.56
Berger-Parker Index (d)	0.95	0.82	0.77
Dominant species	*P. laevis*	*P. laevis*	*P. laevis*

Table 1.7. Seasonal profile of the diversity characteristics of the total component community.

Sampling sites		Vidin	Kozloduy	Silistra
Spring	H_S	0.34	0.60	0.32
	E	0.25	0.43	0.22
	D	1.21	1.61	1.20
	d	0.91	0.75	0.91
Summer	H_S	0.09	0.59	0.62
	E	0.06	0.37	0.44
	D	1.03	1.58	1.68
	d	0.98	0.76	0.72
Autumn	H_S	0.24	0.22	0.42
	E	0.17	0.16	0.38
	D	1.11	1.10	1.33
	d	0.95	0.95	0.86

1.3.3 Water quality classification

The mean values of nutrient and heavy metal concentrations for the period 2003-2005 adjacent to our sampling sites are summarized in **Table 1.2**. Nutrients such as ammonium-N, nitrite-N, ortho-phosphate and total phosphorus were lowest at the downstream site. Similarly, concentrations of copper (Cu) and lead (Pb) in the upper Danube sites were higher in the period 2004–2005 (ICPDR, 2008*b*), whereas nearly no difference occurred for Cd. Results obtained from the second Joint Danube Survey (JDS2) performed in autumn 2007 revealed the same pattern of pollution and eutrophication parameters between the sampling sites (ICPDR, 2008*a*). Accordingly, no significant change in nutrient and heavy metal levels occurred during our sampling period. Although no taxa lists are available for macrozoobenthos communities all sampling sites were categorised to class II according to the saprobic index (ICPDR, 2002).

1.4 Discussion

The composition of endoparasite communities at the investigated Danube sites were principally similar but showed differences which can be attributed to the local ecological conditions. In general, ten endohelminth species were identified, none of which is a barbel specialist. Two of three parasite species recorded for the first time for barbel were considered as cases of accidental infection. Larvae of *Hysterothylacium* sp. were found in the intestine of a barbel collected at the sampling site Kozloduy. The fish most likely acquired this infection while feeding on crustacean, fish intermediate or paratenic hosts. Various small fishes and invertebrates serve as obligate intermediate or paratenic hosts for the nematode's third stage larvae (Moravec, 1994). Some authors suggest that large barbels feed also on small fishes like bullhead or gudgeon (Moravec *et al*. 1997). A closer look into the digestive system of barbels during dissection confirmed small fishes as part of the diet, especially gobiid specimens (Gobidae) were recovered.

The infection with the acanthocephalan *L. plagicephalus* observed at the sampling site Vidin was based on a single well developed male, found in the gut of a fish sampled in the summer of 2007. The definitive hosts of *L. plagicephalus* are sturgeons (Acipenseridae) and its distribution is restricted mainly to Ponto-Caspian basins and drainages including the Danube river basin, where diverse sturgeon species inhabit. Like its host, *L. plagicephalus* is euryhaline, however it has a fresh water life cycle (Skryabina, 1974). The latter suggests that the barbel might have ingested an intermediate host, infested with this particular

acanthocephalan.

In contrast to the single findings of *Hysterothylacium* sp. and *L. plagicephalus* the nematode *Eustrongylides* sp. occurred with high prevalence and intensity at all sites. Highest infection rates were usually observed in bigger fish, as they feed on small fishes which are used as second intermediate hosts for *Eustrongylides* sp. The barbel serves as a paratenic host for *Eustrongylides* sp., similar to other species of the family cyprinidae (Moravec, 1994). The parasites were located in the anterior part of the body cavity, mainly on the serosa of the intestine and in the liver tissue. In most cases, the larvae were surrounded by a capsule, forming a spiral granuloma, as described by Mihalca *et al*. (2007*a*). Simultaneously, free moving nematodes were found, which appeared to cause massive histological damage such as penetrations of the cavity wall and disruptions of inner organs. Infection with nematodes of the genus *Eustrongylides* was recorded from water dwelling reptiles (Reptilia) from different localities in Romania and from the Danube delta region as well. This parasite occurred with high prevalence and intensity in dice snake (*Natrix tessellata*), sampled in the period 2002 – 2006. The grass snake (*Natrix natrix*) was described as a new host of *Eustrongylides excisus* (Mihalca *et al*. 2007*b*).

The dominant parasite species at all sampling sites was the acanthocephalan *P. laevis*. Similar results were obtained in the upstream part of the Danube River (Moravec *et al*. 1997; Schludermann *et al*. 2003; Laimgruber *et al*. 2005). However, the parasite list of *B. barbus* published by Margaritov (1966) and Kakacheva- Avramova (1977) for the Bulgarian section of the Danube River differs greatly from the parasite fauna detected in the present study. During our study period no cestodes were recovered, although Margaritov (1966) and Kakacheva- Avramova (1977) reported three cestode species (*Caryophyllaeus brachycollis, C. laticeps, C. fennica*) for barbel. The absence of cestodes during our sampling period could be explained with high *P. laevis* infection levels, which result from the barbel's preferred diet consisting of amphipods and small fishes. The feeding habits of barbel and its diet are influenced by the available local invertebrate fauna, which itself is determined by the water quality and habitat composition. A major characteristic of the principal invertebrate fauna in the Danube River is the high abundance of gammarids, from which some species are known to be appropriate intermediate hosts for *P. laevis* (Rumpus and Kennedy, 1974; Marshall, 1976; Moravec and Scholz, 1991; Dezfuli *et al*. 2000). Preferred feeding of fish on amphipods results in high abundance of *P. laevis*, which obviously reduces the diversity of parasite communities (Kennedy et. *al*. 1986; Moravec *et. al.* 1997).

The second most frequent parasite at all Danube localities, *R. hellichi*, occurred at the sampling site Vidin with a prevalence of 24.2%. The prevalence was about two times lower

compared to the data obtained from the other two sampling sites. According to Moravec and Scholz (1995) trichopteran larvae from the genus *Hydropsyche* serve as intermediate hosts for the transmission of *R. hellichi* (see e.g. Moravec, 1995). Thus, the lower prevalence at Vidin can be explained with a lower abundance of the intermediate host, which could be correlated to a higher eutrophication and pollution level in this part of the river. The larvae of *Hydropsyche* sp. are well established indicators which are used to assess the water quality (Moog, 1995). For example, the saprobic index of trichopteran larvae varies between 2.1 and 2.3 and corresponds to water quality class 2.

Moreover, the prevalence recorded for the nematode *Eustrongylides* sp. in Vidin was the highest at all sampling sites. This supports the pollution hypothesis, since the first intermediate host described for *Eustrongylides* sp. are aquatic oligochaetes such as *Lumbriculus variegatus* (Lumbriciidae), *Tubifex tubifex* and *Limnodrilus* sp. (Tubificidae) (Moravec, 1994). All these oligochaete species indicate disturbed aquatic habitats (saprobic index over 3, pollution with chemicals) where they are highly abundant.

The results of the present study correspond very well with data of Valtonen *et al.* (1997) who also correlated the occurrence of single parasite species in fish with the abundance of intermediate hosts from lakes with differences in trophic status and degree of pollution. For example the acanthocephalan *Acanthocephalus lucii* showed the highest prevalence in perch (*Perca fluviatilis*) from a eutrophic and polluted lake. The intermediate host of *A. lucii*, *Asellus aquaticus*, is known as pollution tolerant and is highly abundant under contaminated conditions (Murphy and Learner, 1982). Not only the occurrence of a single parasite species can be related to environmental parameters but also the composition and diversity of whole parasite communities is determined by environmental conditions such as eutrophication, pollution and changes in substrate composition. These conditions can either directly affect the parasite (e.g. toxic effects on free-living stages) or indirectly by affecting the abundance and distribution of the respective intermediate and final hosts (Sures, 2004*a*). Evidence from the field revealed the composition of fish helminth communities being largely dependent on the benthic invertebrate fauna, which itself is directly dependent on water quality and benthic habitats (Sures and Streit, 2001; Laimgruber *et al*. 2005; Thielen *et al.* 2007).

In the present study the lowest value for the Brillouin index and the Shannon-Wiener diversity was recorded for Vidin. As parasite diversity is considered a measure of ecosystem health (Hudson *et al*. 2006), the higher diversity at Silistra gives evidence for better environmental conditions in the lower river stretch. This is confirmed by hydrochemical data, which indicate a higher level of pollution and eutrophication at Vidin compared to Silistra. Eutrophication might favour the occurrence of intermediate hosts known to be tolerant against high nutrient

concentrations such as annelids and crustaceans. Additionally, the presence of toxic metals supports the occurrence of parasites transmitted by anneldids or crustaceans for example by compromising the immune system of the definitive host. Thus, the combined effects of high nutrient and pollutant concentrations represent favourable ecological conditions especially for the dominant occurrence of *P. laevis*. This dominance also negatively affects infracommunity and component community diversity as it leads to lower values for the Shannon-Wiener and Simpson index. Our results therefore give good evidence that aquatic ecosystem health could be assessed by investigating the composition and diversity of fish parasite communities, which – also due to their position in food webs (Lafferty *et al*. 2008) – represent an integrative measure of the overall ecological conditions.

2 Is metal accumulation in *Pomphorhynchus laevis* dependent on parasite sex or infrapopulation size?

2.1 Introduction

The increasing industrialization and enhancement of human activities leads to rising levels of contaminants in aquatic habitats. This requires a permanent monitoring of the presence and effects of pollutants. For detecting chemicals in aquatic ecosystems, analytical methods are established and different groups of bioindicators such as bivalves are available (Arndt *et al.* 1987; Reeders *et al.* 1993; Gunkel, 1994). Besides established free-living sentinel species, recent studies suggest that fish parasites might also be useful as monitoring organisms for the detection of chemical pollution (Sures, 2003). Due to their excellent ability to accumulate different substances, intestinal parasites, especially fish acanthocephalans, have been suggested as suitable bioindicators for metal pollution (summarized in Sures, 2003, 2004*a*; Vidal-Martinez *et al.* 2010). The accumulation capacity of acanthocephalans has been shown to exceed even that of established free living sentinel organisms such as the zebra mussel, *Dreissena polymorpha* (see Sures *et al.* 1997*a*, 1999*b*). Accordingly, fish-acanthocephalan systems represent promising monitoring tools for the detection of chemical pollution in aquatic systems, especially if contaminant levels are low, e.g. in pristine areas such as the Antarctic (Sures and Reimann, 2003). For practical reasons the fish host must be widely distributed and easy to be sampled (Kennedy, 1997) in order to use endoparasites and their hosts as bioindicators. Moreover, the parasites have to be highly abundant and prevalent in their host species (Sures, 2004*a*). Promising host-parasite systems for freshwater habitats are cyprinid fish species such as barbel, *Barbus barbus*, infected with the palaeacanthocephalan *Pomphorhynchus laevis* (see Sures, 2004*b*). Different benthic crustacean species serve as intermediate host for *P. laevis* (Rumpus and Kennedy, 1974). As they form a substantial part of the diet of benthivore fishes, high infection intensities are commonly described for fish species such as barbel.

Although several aspects of the mechanism and kinetics of metal uptake in acanthocephalans have been elucidated by Sures (2001), there are still some open questions concerning the applicability of fish parasites as sentinels. For example, the effect of the acanthocephalan infrapopulation size on the process of metal accumulation in fish-parasite systems is not known. Some authors reported that fish parasites are able to reduce element concentrations in their host tissues (Sures and Siddall, 1999), which might correlate with the infrapopulation size (Thielen *et al.* 2004). Moreover, there are no data available concerning metal

accumulation in relation to the sex of fish acanthocephalans, which could also play a considerable role. Indeed, several metabolic pathways are different according to gender in acanthocephalans (Crompton and Nickol, 1985), and previous studies on experimental infections of terrestrial mammals with acanthocephalans provided contradictory results on metal accumulation according to parasite sex (Scheef *et al.* 2000; Sures *et al.* 2000*a*,*b*).

The field study presented in this chapter was designed to address these aspects using the fresh water cyprinid *Barbus barbus*. Barbel is the second largest cyprinid species in Europe and it is wide spread throughout large river systems. It is well known for its high infection levels with the acanthocephalan *Pomphorhynchus laevis* in the Danube River (Kakacheva-Avramova, 1962, 1977; Margaritov, 1959, 1966; Moravec *et. al.* 1997; Schludermann *et al.* 2003; Thielen *et al.* 2004; Laimgruber *et al.* 2005; Nachev and Sures, 2009). Accordingly, the model system *B. barbus- P. laevis* was taken for studying the differences in accumulation with respect to infrapopulation size and the sex of the acanthocephalan.

2.2 Materials and Methods

2.2.1 Sample collection

A total of 27 barbels were collected in September 2006 from local fishermen in the area of Kozloduy, Bulgaria, around river kilometer 685 of the Danube River. After capture, the fish were frozen and transported to the laboratory, where length, weight, sex and age were determined for each fish. The specimens were dissected using standard parasitological techniques. Tissue samples from fish (muscle, intestine and liver) and parasites were taken using stainless steel dissecting tools, which were previously cleaned with 1% ammonium-EDTA solution and double- distilled water to avoid contamination. The sample material was rinsed with physiological solution (0.8% NaCl suprapure) and frozen at -20°C until metal analyses. After collection and identification of the acanthocephalans the following parasitological parameters were calculated according to Bush *et al.* (1997) - prevalence (P, %), intensity range (IR), abundance (A) and mean intensity (MI) of the infection. The fresh weight and sex (distribution of ♂♂ and ♀♀) of the collected acanthocephalans was also recorded.

Fish were divided into groups according to their infection status or sex. The first group (lightly infected group, LI) comprised barbels (n=9) infected with less then 20 acanthocephalans. For the second group (heavily infected group, HI) fish (n=9) showed a mean infection intensity exceeding 100 worms. Another group consisted of fish (n= 8) with an

infection intensity around the mean value (80.2 worms per fish) obtained for all collected fishes. This group was used to compare a sex specific metal accumulation.

2.2.2 Molecular identification of Pomphorhynchus laevis

While the parasite species was identified as *P. laevis* using morphological traits, the possibility of co-infection with other closely related acanthocephalan species such as *Pomphorhynchus tereticollis* persists, due to their similarity in morphology, biology and transmission (Perrot-Minnot, 2004). Therefore, a molecular method was used for parasite identification. DNA was extracted from 200 parasites taken randomly from different fishes following the procedure described by Franceschi *et al.* (2008). Species identification was performed using a diagnostic PCR to amplify a portion of the internal transcribed spacer (ITS) rDNA gene (see Franceschi *et al.* 2008 for primers and PCR conditions). For each PCR reaction, one negative (reaction solution without template DNA) and three positive controls (template DNA from already-identified *P. laevis, P. tereticollis* and *Polymorphus minutus*) were carried out. The sizes of PCR products determined the parasite species (Franceschi *et al.* 2008): the products for *P. laevis*, *P. tereticollis* and *P. minutus* were 320, 350 and 290 bp, respectively (**Figure 2.1**). The analyses revealed that all acanthocephalans were *P. laevis*, which confirmed the absence of co-infection by different acanthocephalan species (**Figure 2.1**).

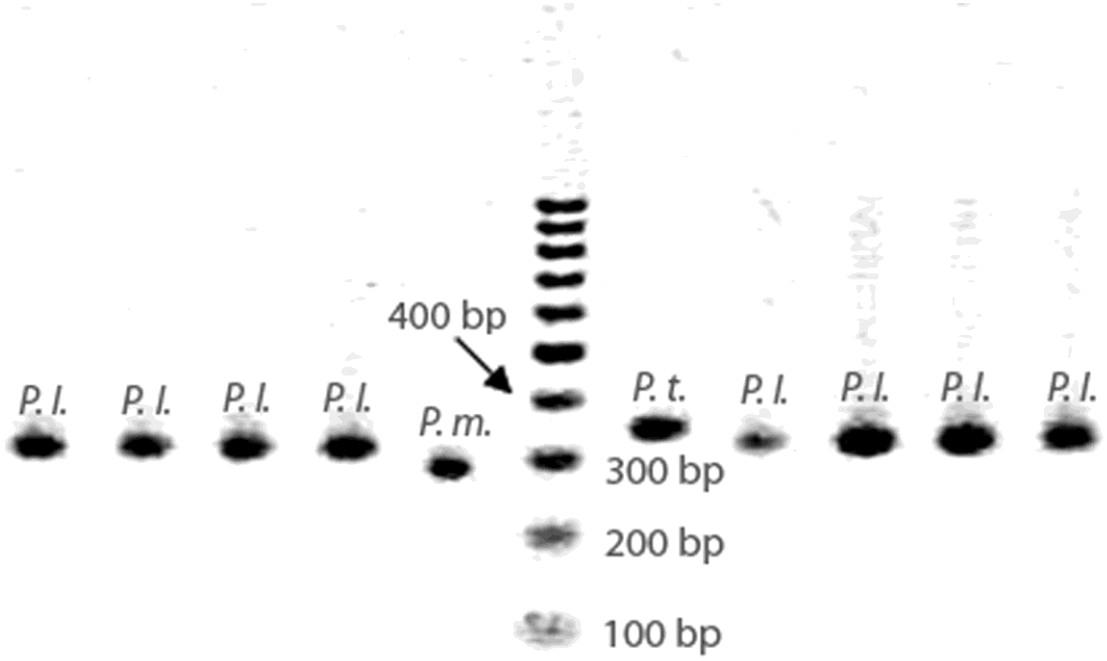

Figure 2.1. Molecular identification of acanthocephalan species, according to the size of PCR product of the partial ITS sequence. The central line is the molecular weight marker.

P. l. – *Pomphorhynchus laevis*; *P. m.* – *Polymorphus minutus*; *P. t.* – *Pomphorhynchus tereticollis*;

2.2.3 Heavy metal analysis

The fish and parasite samples were prepared for analysis using microwave assisted digestion following the procedure described by Zimmermann *et al.* (2001). Up to 300 mg (wet weight) of sample, previously homogenized, was weighed and placed into 150 ml perfluoralkoxy (PFA) vessels, into which a mixture of 1.3 ml nitric acid (65% HNO_3, suprapure) and 2.5 ml hydrogen peroxide (30% H_2O_2, suprapure) was added. Subsequently, the vessels were heated for 90 min at about 170˚C using the microwave digestion system MDS-2000 (CEM GmbH, Kamp-Lintfort, Germany). After digestion the clear sample solution was brought to volume with doubly distilled water in a 5 ml volumetric glass flask and kept in polypropylene sample tubes until analysis.

The concentrations of arsenic (As), cadmium (Cd), colbalt, (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), tin (Sn), vanadium (V) and zinc (Zn) were analyzed using inductively coupled plasma mass spectrometry (ICP-MS). The analyses were carried out with a quadrupole ICP-MS system (Perkin Elmer - Elan 5000)

operating at 1100 W plasma power, 13.3 L/min plasma gas flow, 0.75 L/min auxiliary gas flow and 0.95 L/min nebuliser gas flow and an auto sampler system (Perkin Elmer AS-90) connected with a peristaltic pump with a sample flow of 1 ml/min. To avoid contamination and memory effects the wash time between measurements was set at 10 seconds (with 1% HNO_3, suprapure). Before analyses, the samples were diluted 1:10 using a solution of 1% HNO_3 (suprapure) with a concentration of 10 ng/L of yttrium (Y) and thulium (Tm) as internal standards. In order to control the accuracy and stability during measurements a standard solution (ICP Multielementstandard IV solution, Merck, Darmstadt, Germany) was analyzed after every 10 samples.

The calibration was carried out with a series of 11 dilutions of a standard solution (ICP Multielementstandard solution, Merck, Darmstadt, Germany). Element concentrations were calculated as mg L^{-1} using corresponding regression lines (correlation factor ≥ 0.999). To check the accuracy of the analytical procedure, standard reference material (DORM-3, National Research Council, Canada) of dogfish (*Squalus acanthias*) was analyzed and the values of 10 certified elements were checked. Detection limits for the investigated elements were calculated as the three fold standard deviation of concentrations found in 12 procedural blanks.

2.2.4 Data analyses and statistical treatment

Bioconcentration factors were calculated according to Sures *et al.* (1999*a*) as follows: ($C_{[P.laevis]}$ / $C_{[host\ tissue]}$). If tissue concentrations for one element were below the respective detection limit, the detection limit was used to calculate the bioconcentration factor.

As our data did not meet conditions for parametric analyses, even after transformation, non parametric tests were applied. For comparisons of element concentrations in tissues and *P. laevis* between heavily infected and lightly infected barbels a Mann-Whitney *U*-test was used with a significance level of $p \leq 0.05$. Wilcoxon matched pair test was applied to determine differences between element concentrations of females and males as well as between fish tissues and the parasites. All statistical tests were performed using STATISTICA 6.0.

2.3 Results

2.3.1 Fish samples

The mean (± S.D.) weight and size of the barbels was 376 ± 139 g and 34.6 ± 4.9 cm, respectively. The age varied between 2 and 5 years, whereas most fishes were 3-4 years old. Only one out of all collected fish was not infected with the acanthocephalan *P. laevis*. This fish was not considered in the following analyses. As expected, the parasite occurred with a high level of infection (P 97.1%, MI 80.2 and A 77.9).

2.3.2 Analytical procedure

Detection limits and mean concentrations of elements in the reference material (DORM-3) are listed in **Table 2.1**. For eight metals present in the standard reference material accuracy rates ranging between 87% to 106% were obtained with the highest accuracy for iron (100%).

Table 2.1. Trace metal concentrations in Dogfish Muscle Certified Reference Material (DORM 3), accuracy and detection limits determined by ICP-MS analyses.

Element	DORM-3 values ± SD (mg/kg)	DORM-3 measured ± SD (mg/kg)	Accuracy (%)	Detection limit (µg/L)
As	6.88 ± 0.3	6.30 ± 0.4	92%	0.008
Cd	0.29 ± 0.02	0.27 ± 0.02	94%	0.01
Co	n.c.	-	-	0.009
Cu	15.5 ± 0.63	16.35 ± 0.93	105%	0.19
Fe	347 ± 20	346.95 ± 28.24	100%	2.76
Mn	n.c.	-	-	0.1
Mo	n.c.	-	-	0.02
Ni	1.28 ± 0.24	1.21 ± 0.15	94%	0.47
Pb	0.395 ± 0.05	0.417 ± 0.04	106%	0.26
Sn	0.066 ± 0.012	0.0067 ± 0.010	102%	0.01
V	n.c.	-	-	0.01
Zn	51.3 ± 3.1	44.4 ± 3.2	87%	2.77

n.c.: element not certified

2.3.3 Element concentrations in barbel and Pomphorhynchus laevis

The concentrations of the five elements As, Cd, Cu, Pb and Zn were found to be significantly higher in the acanthocephalan *P. laevis* when compared with the host tissues (**Figure 2.2** and **Table 2.2**). With the exception of Sn, the levels of all other metals were higher in the parasite than in the muscle of barbel. The element Sn was below or close to the detection limit in the parasites, whereas the levels in the host were significantly higher.

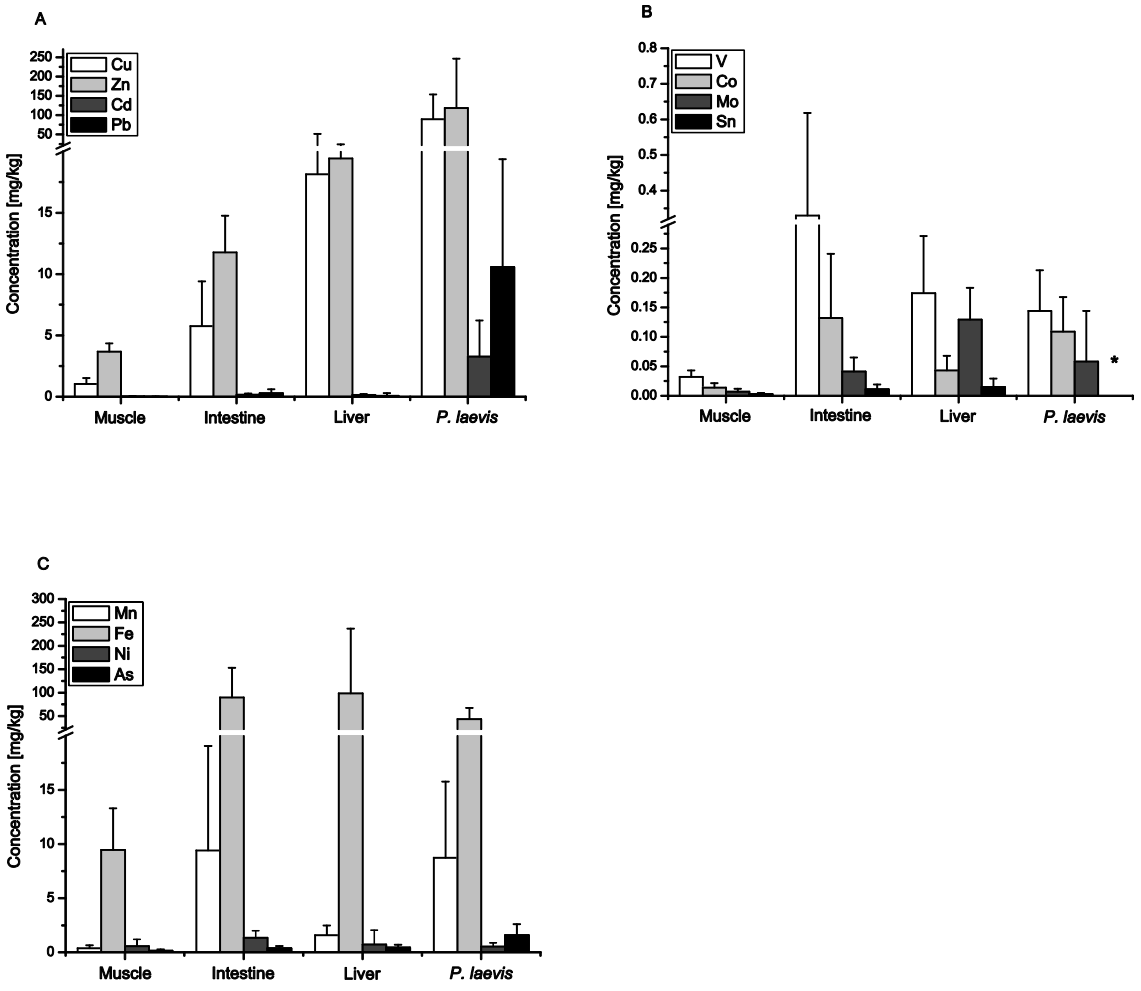

Figure 2.2. Mean (± SD) element concentrations (a-c) in organs of barbels and its intestinal parasite *Pomphorhynchus laevis*. *Concentrations of Sn in *P. laevis* samples are not displayed as they were below the detection limit.

Comparisons of metal concentrations among the fish tissues showed only one clear pattern: the concentrations of all elements were lowest in the muscle (with the exception of Sn). Else, some elements such as Cu, Mo and Zn, were present at higher concentrations in the liver; others such as Co, Mn and Pb were present at significantly higher levels in intestinal samples.

Table 2.2. Differences between element concentrations in barbel organs and *Pomphorhynchus laevis*.

Element	P.l. ↔ M	P.l. ↔ I	P.l. ↔ L	M ↔ I	M ↔ L	L ↔ I
As	P.l**	P.l**	P.l.**	I**	L**	n.s.
Cd	P.l.**	P.l.**	P.l.**	I**	L**	n.s.
Co	P.l.**	n.s.	P.l.**	I**	L**	I**
Cu	P.l.**	P.l.**	P.l.**	I**	L**	L**
Fe	P.l.**	n.s.	L**	I**	L**	n.s.
Mn	P.l.**	n.s.	P.l.**	I**	L**	I**
Mo	P.l.**	n.s.	L*	I**	L**	L**
Ni	n.s.	I**	n.s.	I**	n.s.	n.s.
Pb	P.l.**	P.l.**	P.l.**	I**	L**	I*
Sn	n.t.	n.t	n.t	I*	L**	n.s.
V	P.l.**	n.s.	n.s.	I**	L**	n.s.
Zn	P.l.**	P.l.**	P.l.**	I**	L**	L**

M: Muscle; I: intestine; L: liver; P.l.: *Pomphorhynchus laevis*

*: significant at $p \leq 0.05$ (Wilcoxon matched pair test)

**: significant at $p \leq 0.01$ (Wilcoxon matched pair test)

n.t.: not tested as concentration in *Pomphorhynchus laevis* was below the detection limit.

n.s.: not significantly different (Wilcoxon matched pair test)

In case of significant difference, the site for higher concentration is given in each cell.

The mean bioconcentration factors revealed, that 8 elements (As, Cd, Co, Cu, Mn, Pb, V, Zn) were overall present in higher levels (BCF>1) in the parasites (**Table 2.3**). The metal accumulation capacity of *P. laevis* with respect to host muscle in decreasing order was as follows: Pb> Cd> Cu> Zn>As> Mn> Co> V> Mo> Ni. Nearly the same pattern was observed for the intestine and liver, with Pb> Cd> Cu> Zn> As> Mn> Co> V for the intestine and Pb> Cd> Cu> Zn> As> Mn> Co> Ni> V, for the liver. The remaining elements were detected only in low concentrations in the parasite samples (see **Table 2.3**). The mean concentration factors were found to be up to 1070 times higher for Pb and 195 times higher for Cd compared to the host tissues. The ratios for As, Cu and Zn showed the same tendency with the highest mean values of 12, 95 and 32, respectively.

Table 2.3. Bioconcentration factors $C_{[P.laevis]}$ / $C_{[barbel\ tissue]}$ for *Pomphorhynchus laevis* calculated with respect to different host tissues.

Element	Muscle	Intestine	Liver
	$C_{[P.laevis]}$ / $C_{[Muscle]}$ ±SD	$C_{[P.laevis]}$ / $C_{[Intestine]}$ ±SD	$C_{[P.laevis]}$ / $C_{[Liver]}$ ±SD
As	12.0 (± 9.5)	4.5 (± 3.3)	3.8 (± 3.3)
Cd	194.8 (± 142.8)	22.3 (± 11.9)	23.4 (± 18.7)
Co	8.9 (± 4.6)	1.2 (± 0.7)	2.6 (± 0.8)
Cu	94.7 (± 66.2)	17.8 (± 11.0)	9.6 (± 10.6)
Fe	4.9 (± 3.04)	0.7 (± 0.5)	0.7 (± 0.3)
Mn	22.9 (± 16.2)	1.7 (± 1.6)	5.4 (± 2.6)
Mo	4.7 (± 3.1)	0.9 (± 0.5)	0.3 (± 0.4)
Ni	1.9 (±2.2)	0.5 (± 0.3)	2.2 (± 2.1)
Pb	1070.5 (± 781.8)	81.7 (± 88.5)	433.4 (± 602.4)
Sn	n.d.	n.d.	n.d.
V	4.8 (± 3.0)	0.7 (± 0.5)	1.2 (± 0.9)
Zn	32.2 (± 34.0)	10.5 (± 10.8)	6.4 (± 6.9)

n.d.: concentrations for *Pomphorhynchus laevis* below detection limit

2.3.4 Accumulation differences with respect to parasite infra population size and sex

In general, comparisons between the element concentrations of lightly and heavily infected barbels showed no significant differences (**Table 2.4**). Concerning the parasites, the only significant differences were found for V, with higher levels in the heavily infected group. Fish liver of this group also contained significantly higher vanadium amounts (see **Table 2.4**). In contrast, the concentrations of Cd detected in the intestinal tissue were significantly higher in the lightly infected group (**Table 2.4**).

Comparisons of bioconcentration factors calculated for the metals present in significantly higher levels in *P. laevis* (As, Cd, Cu, Pb, Zn), showed no differences with respect to infrapopulation size. Lightly infected fishes displayed more variation in the values obtained for muscle tissue. However, the pattern of distribution, as well as the ratio ranges for other organs was similar (see **Figure 2.3** and **Figure 2.4**).

When comparing element concentrations with respect to parasite sex, similar values were found for females and males of *P. laevis*. Only the essential elements Zn and V were detected in significantly higher concentrations in the females (see **Table 2.4**).

Table 2.4. Differences in element concentrations between heavily infected (HI) and lightly infected (LI) barbels, as well as between male and female *Pomphorhynchus laevis*.

Element	P.l.(HI) ↔ P.l.(LI)[a]	P.l.♂ ↔ P.l.♀[b]	M(HI) ↔ M(LI)[a]	I(HI) ↔ I(LI)[a]	L(HI) ↔ L(LI)[a]
As	n.s.	n.s.	n.s.	n.s.	n.s.
Cd	n.s.	n.s.	n.s.	I(LI)*	n.s.
Co	n.s.	n.s.	n.s.	n.s.	n.s.
Cu	n.s.	n.s.	n.s.	n.s.	n.s.
Fe	n.s.	n.s.	n.s.	n.s.	n.s.
Mn	n.s.	n.s.	n.s.	n.s.	n.s.
Mo	n.s.	n.s.	n.s.	n.s.	n.s.
Ni	n.s.	n.s.	n.s.	n.s.	n.s.
Pb	n.s.	n.s.	n.s.	n.s.	n.s.
Sn	n.t.	n.s.	n.s.	n.s.	n.s.
V	P.l.(HI)*	P.l.♀**	n.s.	n.s.	L(HI)*
Zn	n.s.	P.l.♀*	n.s.	n.s.	n.s.

M: muscle; I: intestine; L: liver; P.l.: *Pomphorhynchus laevis*

*: significant at $p \leq 0.05$

**: significant at $p \leq 0.01$

[a]: Mann-Whitney *U*-test

[b]: Wilcoxon matched pair test

n.s.: not significantly different

n.t.: not tested

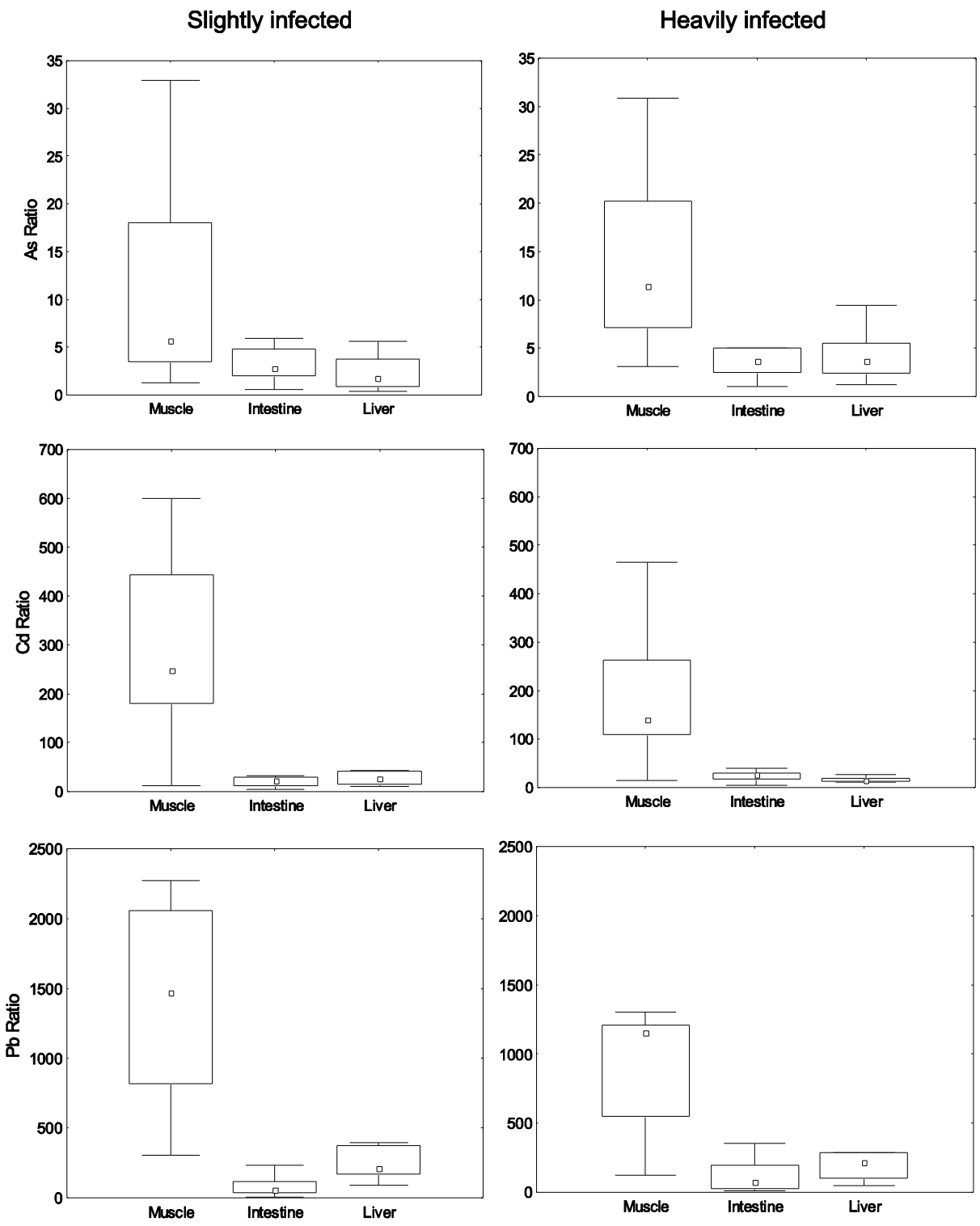

Figure 2.3. Comparisons of the ratios $C_{[P.laevis]}$ / $C_{[\text{organ barbel}]}$ obtained for the toxic elements arsenic, cadmium and lead between heavily and lightly infected barbels. Dots are medians, boxes are interquartile ranges and error bars are interdecile ranges.

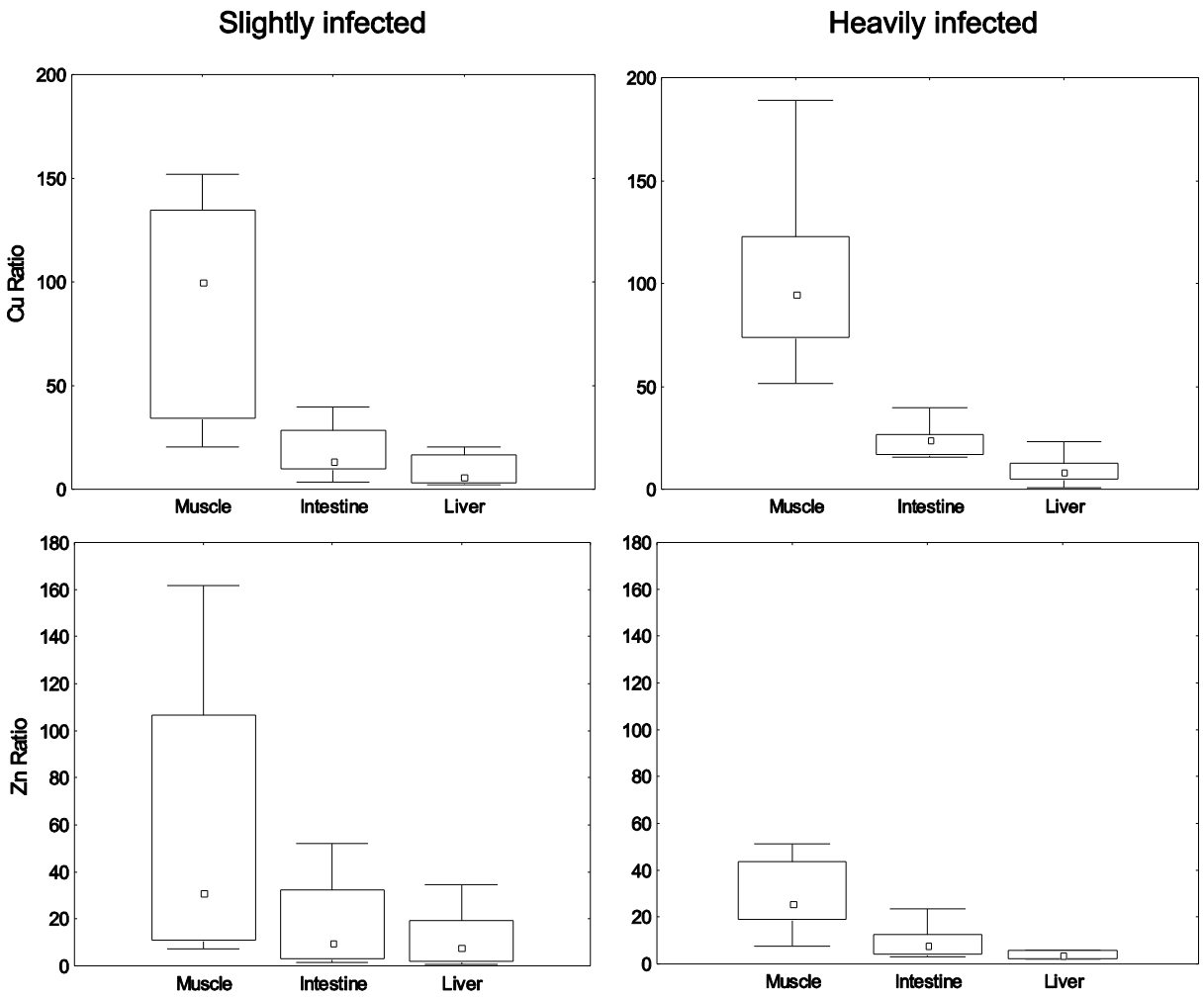

Figure 2.4. Comparisons of the ratios $C_{[P.laevis]}$ / $C_{[organ\ barbel]}$ obtained for the essential elements copper and zinc between heavily and lightly infected barbels. Dots are medians, boxes are interquartile ranges and error bars are interdecile ranges.

2.4 Discussion

As expected and previously reported in various studies on metal accumulation in the host-acanthocephalan system (reviewed by Sures, 2003, 2004*b*), many of the analyzed elements were found in higher concentrations in the acanthocephalan *P. laevis,* compared to its host's tissues. Some toxic and essential elements such as As, Cd, Cu, Pb and Zn were present at significantly higher levels in the parasite. The same results were obtained by Schludermann *et al.* (2003) for Cd, Pb and Zn from a field study in the Austrian part of Danube River. The bioconcentration factors were quite similar in both studies, with the exception of Pb, which was found to be much higher accumulated in *P. laevis* in the present study. The present lead concentrations confirmed previous results of an investigation on chub naturally infected with *P. laevis* from the River Ruhr in Germany (Sures *et al.* 1994*a*). In the latter study mean Pb

values were found to be up to 2700 times higher in the parasite compared with muscle and 770 and 280 times higher than liver and intestine, respectively. The mean Pb concentrations in the fish organs were similar to those obtained during the present investigation, only the amounts detected in the parasite were higher. This discrepancy could be due to differences in lead levels of the river Ruhr and Danube. For example, the average concentration of lead in the water of the river Ruhr was 5 µg/L (Umweltbundesamt, 1992) whereas Pb concentrations in the Danube river near the fish sampling site varied between 2.3 and 2.8 µg/L for the period of 2003-2005 (TNMN, 2009). The mean bioconcentration factors calculated with respect to fish muscle differ in the same order as the water concentrations. Whereas the levels in host tissues were comparable, the acanthocephalans showed a higher Pb accumulation. This supports the use of *P. laevis* as an accumulation indicator due to its sensitive and linear response to aqueous metal concentrations, which was also demonstrated in laboratory exposure studies (Sures, 2004*b*).

With respect to parasite infrapopulation size, the study revealed that the intensity of infection usually plays no significant role for metal accumulation in the host-parasite system. Almost all elements were found in both groups (LI and HI) in similar concentrations in the parasite and host tissues. Accordingly, in general metal accumulation is not influenced by the parasite infrapopulation size. The only significant difference was found for the element vanadium in *P. laevis*. Recently, laboratory studies demonstrated that infections with acanthocephalans can reduce heavy metal burdens in the host (Sures and Siddall, 1999; Sures *et al.* 2003) when comparing uninfected fish with infected conspecifics. Due to a lack of a sufficient number of uninfected fish in the present study, these laboratory findings could not be proven under field conditions.

A further aspect needing clarification in order to use acanthocephalans as accumulation indicators is the evaluation of possible effects of parasite sex on metal accumulation. Until now, there are only few studies based on acanthocephalans of terrestrial mammals that provide data on differences in element accumulation patterns between male and female worms. Scheef *et al.* (2000) and Sures *et al.* (2000*b*) reported higher concentrations of lead and cadmium in females of the acanthocephalan *Moniliformis moniliformis* in experimentally infected and subsequently metal exposed rats. In contrast, element levels analyzed by Sures *et al.* (2000*a*) in pigs naturally infected with *Macracanthorhynchus hirudinaceus* showed the opposite tendency- male worms contained higher concentrations for each element. Accordingly, the low number of publications and the contradictory results do not allow general conclusions. Additionally the mechanism of metal uptake in the fish-acanthocephalan system differs from that of terrestrial mammals. According to previous studies,

acanthocephalans in freshwater fish are exposed to bile bound metals in the small intestine (Sures and Siddall, 1999), after metals were taken up by fish gills, transported into the liver and then excreted with the bile into the intestine (Hofer and Lackner, 1995; reviewed by Sures, 2001). In contrast, excretion of metals in mammals occurs mainly through the kidneys (Merian, 2004). Therefore data from studies on terrestrial mammals might be completely different from results obtained from fish hosts. Considering the results of the present chapter, no clear evidence exists for a possible impact of parasite sex on heavy metal uptake. The only metals found at significantly higher concentrations in females were V (at $p < 0.05$) and Zn (at $p \approx 0.05$), from which at least the latter element can be considered essential and is therefore regulated by the fish. Concentrations of other elements and especially those of potential importance in metal monitoring surveys were not significantly different when comparing both sexes.

The absence of differences for most element concentrations with respect to parasite sex as well as with respect to infrapopulation size together with the enormous accumulation capacity underline the possible use of *P. laevis* as an excellent sentinel for metal pollution. The importance of detecting metals with toxic effects on biota persists due to their adverse effects on the functionality of aquatic ecosystems (Merian, 2004). Furthermore, the EC-Water Framework Directive (WFD, 2000) has the objective of a good chemical quality status for all European waters. Accordingly, pollutant levels in surface waters have to be monitored to decide if their concentrations meet environmental quality standards. In order to use host-parasite systems as bioindicators to detect metal pollution, the contribution of all possible factors, which might affect metal uptake in the system should be elucidated in detail. In this context the present chapter evaluated possible influences of parasite sex and infrapopulation size and the results re-verified the acanthocephalan *P. laevis* as a highly suitable accumulation indicator, which meets all criteria suggested for ideal sentinels (see Beeby 2001; Sures, 2004*a*).

3 Seasonal differences of metal accumulation in *Pomphorhynchus laevis* and its definitive host *Barbus barbus*

3.1 Introduction

Despite a large number of studies on metal accumulation in different host-parasite systems (Vidal-Martinez *et al.* 2010), information about the influence of possible seasonal dynamics on the metal uptake by parasites is still missing. Usually, authors focused on studying the kinetics and metabolism of different heavy metals (Sures and Siddall, 1999; Sures *et al.* 2003) or concentrated on the parasite's accumulation capacity (reviewed by Sures 2001, 2004*a*). However, a seasonal variation of metal concentrations in parasites might exist, connected with the parasite's transmission cycle during the year. The development of fish acanthocephalans, especially of paleacanthocephala, is characterized by typical annual infection patterns of intermediate and definitive hosts (Kennedy, 2006). These patterns can be related to water temperature changes and are, therefore, season and climate dependent (Kennedy, 1985). Moreover, the lifespan of most fish acanthocephalans in the intestine of the definitive host (in which metal uptake of the parasites occurs) does usually not exceed a period of some months (Kennedy, 2006). Regarding the life cycle of *Pomphorhynchus laevis* it is obvious that its development in the gut of the definitive host is formally separated into two phases, which belong to different seasons of the year. The first phase begins immediately after the infection of the fish and is characterized by an enormous growth of the worms. It continues until the parasite is fully mature. The second phase covers the time from the release of eggs until the death of the acanthocephalan. Therefore, a seasonal analysis of metal concentrations in a host-parasite system would help us to elucidate key aspects of the metal uptake process. For example, metal accumulation in host-parasite systems could be affected by the age structure of the parasite infrapopulation or by seasonal changes of host physiology during the year. The latter might be a result of differences in fish activity and metabolism during the cold season (winter). Changes in host activity are expected to have a considerable impact on the physiology of the parasite. Although only few data are available for longevity, fecundity and patency periods, it is known that fish acanthocephalans are able to survive seasonal periods of host starvation in contrast to acanthocephalans from homeothermic hosts such as mammals (Kennedy 2006).

All above mentioned aspects have to be considered if parasites such as acanthocephalans are taken for metal monitoring purposes. Additionally, following the seasonal dynamics of heavy metal concentrations could be also a good approach for rough estimation of lifespan of

P. laevis in its definitive host. Such information is still missing as it is often impossible to examine the fish faeces qualitatively and quantitatively (Kennedy 2006).

The aim of the present chapter was to analyse if a seasonal pattern of metal distribution in a host-parasite system exists. The fresh water cyprinid *Barbus barbus* and its intestinal parasite *P. laevis* were taken as a model system, since they are already described as an appropriate system for environmental metal monitoring (Sures 2004*b*).

3.2 Materials and Methods

3.2.1 Fish samples

Barbels were collected in a seasonal manner (spring, summer, autumn) during the year 2006. The fish were caught by local fishermen near to the town Kozloduy, which is situated at river kilometer 685 of the Danube River. After killing, fish were kept frozen until parasitological examination in the laboratory.

For metal analyses eight barbels from each season were selected with similar morphological characteristics such as body size and weight (see **Table 3.1**). Moreover fish with extremely hight or low levels of infection were excluded in order to reduce the possible effects of different infrapopulation sizes even though the intensity of infection plays no considerable role in the metal uptake process (for details see **Chapter 2**), at least it has no effects on the element concentrations in worms.

The fish were dissected using standard parasitological techniques, whereas the dissecting tools were previously cleaned with 1% ammonium-EDTA solution and double-distilled water to avoid contamination. The acanthocephalans as well as the fish tissues (muscle, intestine and liver) were placed in plastic tubes and were kept frozen until metal analyses.

Table 3.1. Morphological data of barbel.

	Spring (n=8)	Summer (n=8)	Autumn (n=8)
Weight ± SD (g)	430.9 (± 86)	525 (± 243)	373.8 (± 134.7)
Total Lenght ± SD (cm)	36.5 (± 2.7)	37.4 (± 6.3)	35.5 (± 7.2)
Standard Lenght ± SD (cm)	29.9 (± 2.2)	31.1 (± 5.3)	28.4 (± 4.2)
Body Height ± SD (cm)	7.2 (± 0.2)	7.6 (± 1.2)	6.9 (± 0.9)
Condition Factor ± SD	0.88(± 0.09)	0.95 (± 0.09)	0.86 (± 0.22)

3.2.2 Analytical procedure

Up to 300 mg (wet weight) of fish tissue (muscle, intestine and liver) and parasite samples were weighed and prepared for metal analysis using a microwave assisted digestion following the procedure described by Zimmermann *et al.* (2001). After digestion the clear sample solutions were analyzed using inductively coupled plasma mass spectrometry (ICP-MS) and the concentrations of arsenic (As), cadmium (Cd), colbalt, (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), vanadium (V) and zinc (Zn) were determined (for details see **Chapter 2**).

3.2.3 Data analyses and statistical treatment

Collected acanthocephalans were counted and the wet weight of each infrapopulation was recorded. The mean individual weight was calculated after dividing the total infrapopulation weight by the total number of worms.

For the comparisons of metal concentrations among the host tissues and the parasites arithmetic means with standard deviations were calculated. Additionally, in order to express the accumulation capacity of *P. laevis*, the mean bioconcentration factors (BCF) for the different organs were obtained as described by Sures *et al.* (1999*a*) - $C_{[P.laevis]}$ / $C_{[\text{host tissue}]}$. The Wilcoxon matched pairs test was used to compare element concentrations in host tissues and parasites. The element concentrations between the seasons were compared with the Mann-Whitney *U*-test and the *t*-test. Spearman rank correlation coefficients were determined between the mean individual weight and the number of parasites with metal concentrations.

3.2.4 Element concentrations in the Danube River

The concentrations of the elements As, Cd, Cu, Pb and Zn in the water are shown in **Table 3.2** (ICPDR, 2009). The data represent two Danube sites - one (Novo Selo, 845 km) was situated upstream from our fish sampling locality and the other one (Iskar, 637 km) was located about 40 river kilometers downstream. The mean concentrations for the years 2005 and 2006 at both sites were similar, which indicates that the concentrations at site Kozloduy might not differ as well. The data also showed no clear sings for some incidental contamination (hot spots) with metals in the period of 2005-2006.

Table 3.2. Element concentrations in water at two different sites from the Danube River in Bulgaria. (ICPDR, 2009)

Elements (µg/L)	Novo Selo (845 km) (160 km upstream of Kozldoduy)		Iskar (637 km) (40 km downstream of Kozloduy)	
	2005	2006	2005	2006
As	2.2	2.275	2.692	2.382
Cd	0	0	0*	0
Cu	17.5	23.5	5.15	6.1
Pb	0*	0	2.767	2.364
Zn	22.0	20.92	29.73	20.0

* Data regards the second half of the year

3.3 Results

3.3.1 Analytical procedure

The detection limits of the analyzed elements as well as the obtained concentrations for reference material (DORM 3) are listed in **Table 2.1**. The recovery of elements in dog fish material ranged from 87% to 106%.

3.3.2 Element concentrations in fish tissues and parasite samples

The mean element concentrations obtained from the analyzed fish material are presented in **Table 3.3**. The amounts of As, Cd, Cu, Pb and Zn were found to be significantly higher in *P. laevis* than in the host tissues. This trend was also clearly visualized by the calculated mean bioconcentration factors for each tissue (**Table 3.4**). As expected, the lowest concentrations were observed in muscle tissue, which corresponded to the highest calculated values of BCF. In general, the parasites demonstrated their enormous capacity to accumulate lead. The mean lead concentrations in *P. laevis* obtained in spring, for instance, were 1194 times higher compared to those in muscle tissue and 78 and 211 times higher than in intestine and liver, respectively. Cadmium showed a similar pattern, but with lower bioconcentration factors (see **Table 3.3** and **Table 3.4**). Concerning the elements As, Cu and Zn, the order differed slightly, due to the higher metal contents in the liver compared to the intestine.

Reviewing the other analyzed elements, the acanthocephalans demonstrated overall a higher accumulation capacity compared to the muscle tissue. For other organs such a clear tendency was not obvious.

Table 3.3. Seasonal profile of mean (± SD) element concentrations in different tissues of barbel and in *P. laevis*.

Season		Spring	Summer	Autumn
As	Muscle	0.28 (±0.25)	0.21 (±0.15)	0.19 (±0.10)
	Intestine	0.52 (±0.32)	0.35 (±0.20)	0.45 (±0.17)
	Liver	0.67 (±0.42)	0.54 (±0.49)	0.56 (±0.21)
	P. laevis	1.08 (±0.54)	1.01 (±0.60)	1.92 (±1.23)
Cd	Muscle	0.02 (±0.03)	0.01 (±0.01)	0.02 (±0.01)
	Intestine	0.24 (±0.31)	0.11 (±0.07)	0.14 (±0.05)
	Liver	0.16 (±0.22)	0.08 (±0.04)	0.16 (±0.10)
	P. laevis	2.40 (±2.09)	1.34 (±0.55)	2.63 (±0.58)
Co	Muscle	0.02 (±0.01)	0.02 (±0.01)	0.02 (±0.01)
	Intestine	0.15 (±0.08)	0.17 (±0.14)	0.19 (±0.16)
	Liver	0.04 (±0.01)	0.04 (±0.01)	0.05 (±0.03)
	P. laevis	0.07 (±0.02)	0.10 (±0.07)	0.13 (±0.08)
Cu	Muscle	1.45 (±1.36)	1.57 (±1.29)	1.00 (±0.39)
	Intestine	7.37 (±4.87)	4.79 (±2.44)	6.23 (±2.85)
	Liver	16.92 (±11.59)	13.40 (±8.11)	11.76 (±5.90)
	P. laevis	75.37 (±35.43)	53.13 (±24.13)	77.40 (±35.84)
Fe	Muscle	13.29 (±3.54)	10.21 (±4.72)	9.83 (±4.10)
	Intestine	84.55 (±42.22)	70.92 (±17.87)	118.19 (±89.73)
	Liver	71.88 (±23.11)	81.34 (±39.42)	67.94 (±29.24)
	P. laevis	76.62 (±69.21)	41.27 (±23.28)	37.59 (±10.37)
Mn	Muscle	0.37 (±0.15)	0.35 (±0.11)	0.45 (±0.42)
	Intestine	4.44 (±1.48)	7.35 (±6.37)	13.84 (±14.64)
	Liver	1.26 (±0.33)	1.49 (±0.77)	1.45 (±0.80)
	P. laevis	4.67 (±0.85)	7.64 (±5.87)	8.94 (±6.87)
Mo	Muscle	0.01 (±0.004)	n.d.	0.01 (±0.01)
	Intestine	0.06 (±0.04)	0.06 (±0.02)	0.03 (±0.02)
	Liver	0.21 (±0.11)	0.28 (±0.16)	0.13 (±0.06)
	P. laevis	0.05 (±0.02)	0.10 (±0.06)	0.05 (±0.06)
Ni	Muscle	1.26 (±1.31)	0.66 (±0.61)	0.31 (±0.16)
	Intestine	1.58 (±0.74)	2.28 (±1.16)	1.60 (±0.84)
	Liver	0.33 (±0.22)	0.21 (±0.15)	0.28 (±0.15)
	P. laevis	2.73 (±1.30)	0.55 (±0.28)	0.58 (±0.26)
Pb	Muscle	0.01 (±0.01)	0.004 (±0.003)	0.01 (±0.01)
	Intestine	0.09 (±0.03)	0.12 (±0.10)	0.48 (±0.53)
	Liver	0.03 (±0.02)	0.03 (±0.02)	0.06 (±0.05)
	P. laevis	6.83 (±4.87)	5.19 (±3.74)	9.81 (±4.63)
V	Muscle	0.04 (±0.01)	0.04 (±0.01)	0.03 (±0,012)
	Intestine	0.15 (±0.05)	0.29 (±0.36)	0.48 (±0.398)
	Liver	0.12 (±0.05)	0.17 (±0.16)	0.14 (±0.094)
	P. laevis	0.07 (±0.01)	0.10 (±0.04)	0.16 (±0.069)
Zn	Muscle	4.71 (±1.49)	4.76 (±1.51)	3.66 (±0.69)
	Intestine	11.39 (±3.86)	10.40 (±1.16)	10.14 (±1.87)
	Liver	18.82 (±3.37)	19.23 (±5.84)	18.00 (±3.96)
	P. laevis	63.91 (±36.39)	34.83 (±16.66)	91.06 (±43.47)

n.d.: concentrations below detection limit

Table 3.4. Seasonal profile of bioconcentration factors $C_{[P.laevis]}$ / $C_{[barbel\ tissue]}$ for *P. laevis* calculated with respect to different host tissues.

		Spring	Summer	Autumn
As	Muscle	3.8	4.8	10.3
	Intestine	2.1	2.9	4.2
	Liver	1.6	1.9	3.4
Cd	Muscle	103.3	132.7	116.7
	Intestine	10.1	11.7	18.3
	Liver	15.3	16.0	16.8
Co	Muscle	4.4	5.7	8.3
	Intestine	0.5	0.6	0.7
	Liver	1.9	2.3	2.8
Cu	Muscle	50.7	33.9	77.2
	Intestine	10.0	11.1	12.4
	Liver	4.4	4.0	6.6
Fe	Muscle	5.8	4.0	3.8
	Intestine	0.9	0.6	0.3
	Liver	1.1	0.5	0.6
Mn	Muscle	12.8	21.9	20.0
	Intestine	1.1	1.0	0.6
	Liver	3.7	5.1	6.2
Mo	Muscle	7.0	5.9	5.0
	Intestine	0.8	1.6	1.2
	Liver	0.2	0.4	0.3
Ni	Muscle	2.2	0.8	1.9
	Intestine	1.7	0.2	0.4
	Liver	8.3	2.6	2.1
Pb	Muscle	1194.4	1250.0	794.4
	Intestine	78.1	42.2	20.2
	Liver	211.3	151.1	158.1
V	Muscle	2.0	2.8	5.2
	Intestine	0.5	0.3	0.3
	Liver	0.6	0.6	1.1
Zn	Muscle	13.6	7.3	24.9
	Intestine	5.6	3.3	9.0
	Liver	3.4	1.8	5.1

3.3.3 Seasonal differences in acanthocephalan's morphology

The calculated mean individual weight for the parasite infrapopulations demonstrated a clear annual pattern (**Figure 3.1**). The mean individual weight obtained in autumn was found to be significantly lower compared to spring and summer, which indicates that the infrapopulations in autumn consisted mainly of young preadult individuals. The comparisons between spring and summer revealed no significant differences, still the acanthocephalan infrapopulations in summer showed a slightly elevated mean individual weight as expressed by the higher

arithmetic mean and median values. Additionally, the correlation analysis between the mean individual weight and the number of parasites revealed no significant associations.

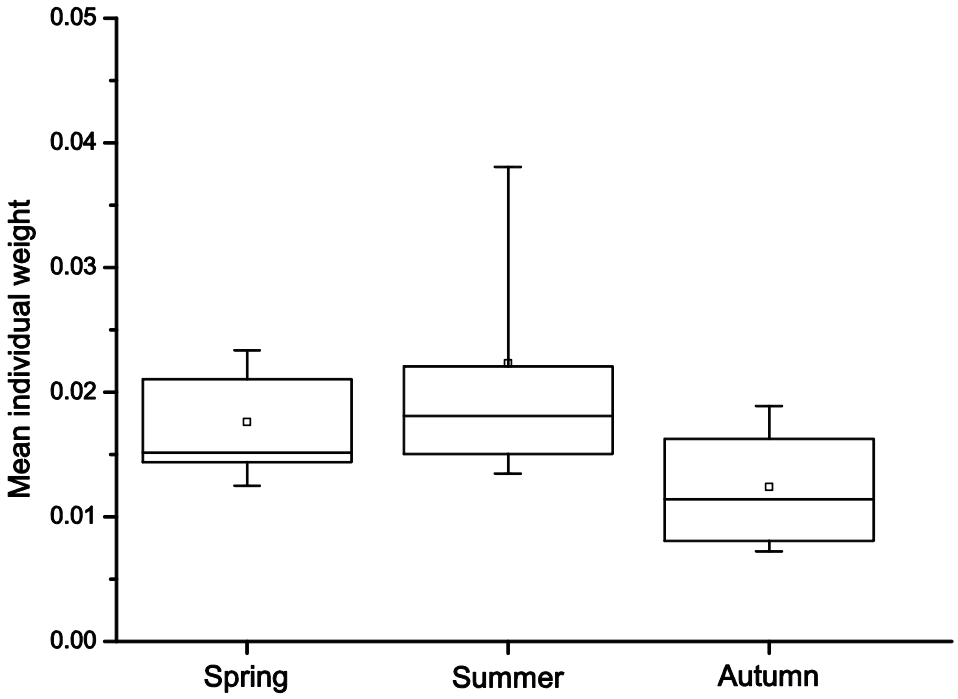

Figure 3.1. Seasonal profile of the mean acanthocephalan weight. Dots are medians, lines are means, boxes are interquartile ranges and error bars are interdecile ranges.

3.3.4 Seasonal variation in concentrations of the elements accumulated by P. laevis

All elements (As, Cd, Cu, Pb, Zn) found with significantly higher concentration in the parasite demonstrated similar seasonal patterns (**Figure 3.2**). Some of them showed a negative correlation with the calculated mean individual weight (e.g. Cd and Pb). Following the annual distribution of these elements, it turned out that they were less concentrated in the worms collected from the barbels sampled in the summer. On the other hand the highest mean concentrations were obtained for autumn, whereas the fish in spring shared concentrations in the range of those obtained for the other seasons (see **Table 3.3** and **Figure 3.2**). Significant differences were found only for Cd and Pb comparing the levels in summer and autumn. The slight variation of mean concentrations obtained for the host tissues during the seasons were also worth mentioning (see **Table 3.3**).

As mentioned above, the concentration of some elements (e.g. Cd and Pb), which were accumulated by the acanthocephalans on a higher level, showed a clear relation to the mean

individual weight of acanthocephalans. In general, a higher mean individual weight corresponded to a lower level of Cd ($R = -0.466$; $p < 0.05$) and Pb ($R = -0.426$; $p < 0.05$). Such a relationship was not observed for As and the essential elements Cu and Zn.

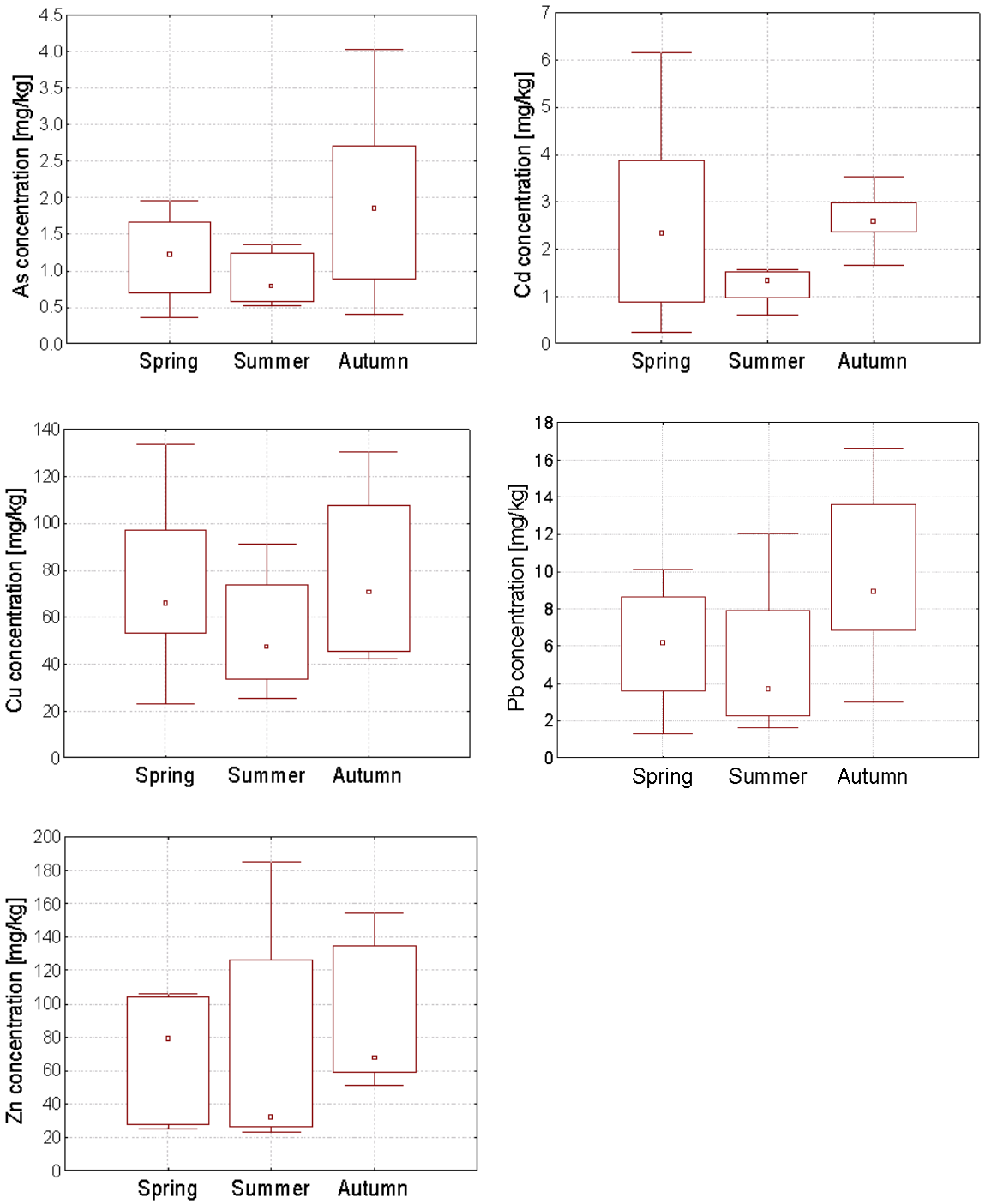

Figure 3.2. Seasonal pattern of the element concentrations accumulated by *P. laevis*. Dots are medians, boxes are interquartile ranges and error bars are interdecile ranges.

3.4 Discussion

The obtained results for the element concentrations in the host-parasite system confirmed similar tendencies described in other metal monitoring studies on acanthocephalans and their fish hosts. For example in a field study, accomplished by Schludermann *et al.* (2003) in the Austrian part of Danube River, the concentrations for Cd, Pb and Zn in *P. laevis* were considerably higher compared to the levels in the barbel's tissues. Similar results were also published by Thielen *et al.* (2004) for fish from the Danube near Budapest after analyzing a wide number of elements. *P. laevis* showed an enormous accumulation capacity (e.g. for lead) in different fish hosts such as chub (Sures and Siddall, 2003). According to all available data, there is no doubt that the acanthocephalans are very useful in terms of metal indication, due to their excellent response to the ambient element levels. Hence, the persent results for As, Cd, Cu, Pb and Zn in *P. laevis* support again the fact that the acanthocephalans are good sentinel organisms.

The observed seasonal pattern of heavy metal concentrations in the parasites could not be related to some incidental contamination (hotspot pollution) in this part of the river. The concentrations of these elements in the water showed no wide variation in the period of 2005-2006 (see **Table 3.2**). In general, the differences in the course of the year could be explained with the seasonality of the acanthocephalan's transmission, more precisely with the stage of development (maturation) in the gut of the final host. Seasonal aspects in the transmission were reported for various aquatic parasites, whereas the climate conditions often played the decisive role. For instance, the oscillations in prevalence of acanthocephalans were assumed to be a consequence of seasonal fluctuation in the temperature of the habitat (Kennedy, 1985). According to Kennedy (2006), if there is a possibility of the intermediate host to breed throughout the year, for instance in some warmer areas like South England, the seasonality of infection levels in the intermediate and final host may not be clearly expressed, since gammarids of all sizes are permanently presented. However, he reported that the prevalence of cystacanths was slightly higher in summer but lower during winter months. The results published by Hine and Kennedy (1974) for the dace (*Leuciscus leuciscus*), as a definitive host, showed no clear seasonal deviation of prevalence, abundance or maturation, while the fish was able to acquire infection throughout the whole year. In contrast to the dace, the barbel's activity differs strongly in terms of water temperature. Its activity budget decreases progressively with the decrease of water temperature and decreasing to the thermal limit for activity (4.0° C) the barbel enters into a dormancy phase (Baras, 1995). The area where the sampling was conducted, is characterized by a typical continental climate in contrast to South

England, whereas the water temperature in winter months (for the period of December to March) was mostly below or around the barbel's thermal limit of activity, the temperature data was provided by TransNational Monitoring Network database (ICPDR, 2009, **Appendix III**). Thus, the reduced fish activity and the correspondingly altered feeding behavior lead probably to a complete reduction of infection during winter months, while the host stops feeding on gammarids. The temperature-related alterations in the host behavior are an important factor in initiating an outbreak of parasites at the beginning of every annual cycle, due to the fact that the transmission proceeds via a predator-prey relationship (Kennedy, 1985). Furthermore, the low temperatures affect the feeding behavior and the reproduction activity of the amphipods, which also may decrease the transmission efficiency of the acanthocephalans. Consequently, the biology of the intermediate host is another important factor, which plays a considerable role in the seasonality of infection. For example, in some areas where *P. laevis* uses *Gammarus duebeni* as intermediate host, a clear seasonality in infection levels of its definitive host *Salmo trutta* was observed (Molloy *et al.* 1995). This was caused by the pronounced seasonal cycle of growth and reproduction of this gammarid (Fitzgerald and Mulcahy, 1983). Thus, the combination of the available literature data and the chemical data acquired in the present study shows that the seasonal pattern of metal accumulation corresponds to the seasonal dynamics of the acanthocephalans transmission (**Figure 3.3**), even though the given data for the prevalence of cystacanths and adults represent the conditions from another geographical region and are related to another final host. In the lower Danube it might be expected that the acanthocephalans exhibit a better defined seasonality of transmission than the observed trends published by Kennedy (1985, 2006).

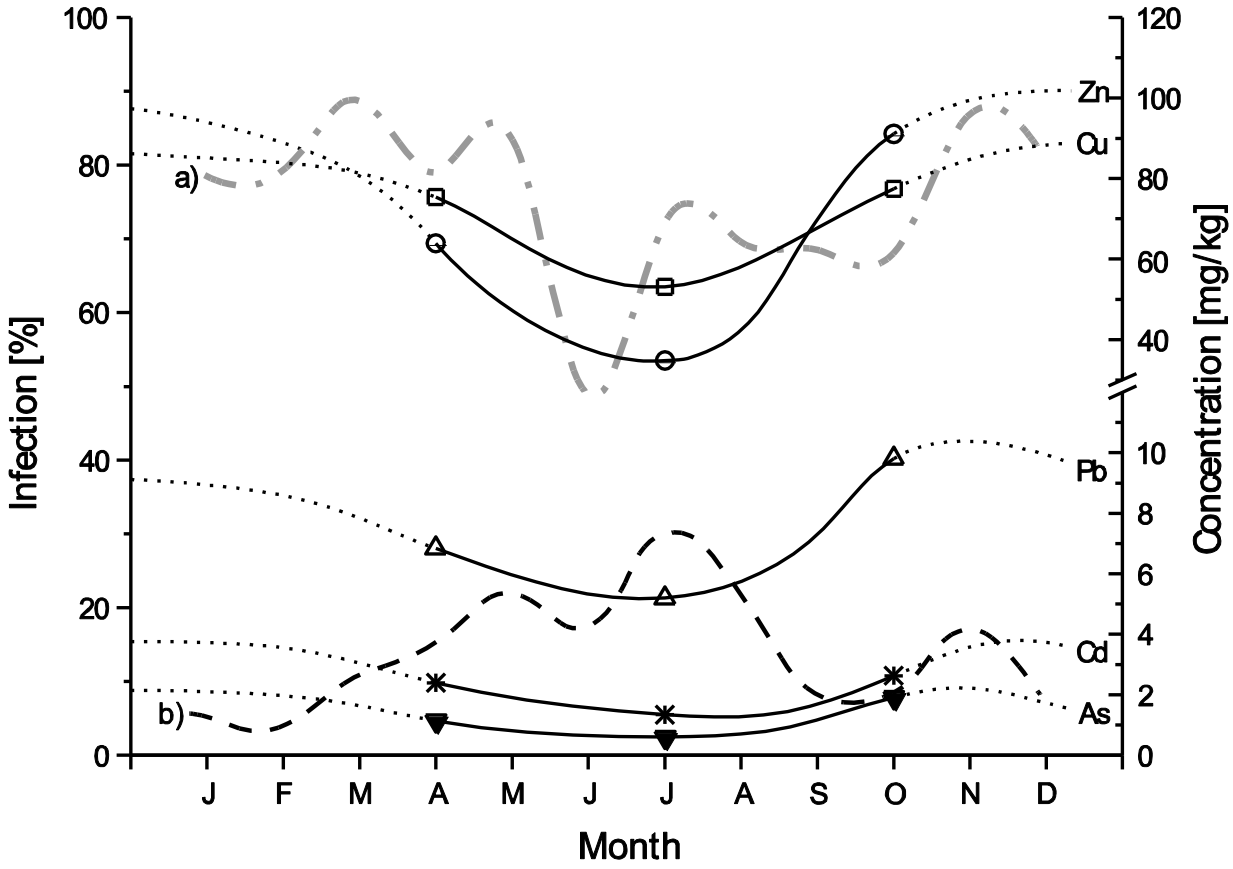

Figure 3.3. Seasonal pattern of the concentrations of the elements As, Cd, Cu, Pb and Zn fitted according to changes in prevalence of (a) adult *P. laevis* in fish and (b) cystacanths in gammarids (Hine & Kennedy, 1974). (From Kennedy, 1985; 2006).

It can be concluded that the acanthocephalan's infrapopulations were characterized by different age compositions in the different seasons covered by the present investigation. The discrepancies obtained for the mean worms' weight in the course of the year (see **Figure 3.1**) are taken as evidence for this. Simultaneously, the concentration patterns of all accumulated elements were similar throughout the year, which was an additional sign that metal uptake was related to the stage of development. Thus, following the development of acanthocephalans with respect to the accumulation process, it seems that in autumn *P. laevis* infrapopulations consisted mainly of young worms, which were suggested to occur in the growth phase (preadults). Therefore, due to accelerated metabolism, as previously mentioned, the obtained mean concentrations of the accumulated elements were on the highest level. An additional evidence for this was the significantly lower mean individual weight obtained for the parasite's infrapopulations in this period compared to the summer. Furthermore, the negative relationship between the concentrations of the elements Cd and Pb and the mean individual weight additionally confirmed the relationship between the accumulation process

and the stage of development (characterized by the mean individual weight) in the gut. A similar tendency was described for other organisms established in metal monitoring like the shellfish, whereas the small individuals are characterized with faster uptake than the larger ones (Strong and Luoma, 1981). The increased surface-volume ratios of the younger (smaller) specimens leads to a higher uptake from the solution (water medium) and causes negative associations between size and concentration. The same statement could be made for the fish acanthocephalans, as the assimilation of nutrients and heavy metals occurs mainly through the worm's tegument.

For spring, it can be suggested that the slowed barbel's metabolism during the cold months affected the metabolism of the acanthocephalan regarding accumulated elements. This lead to a decrease of their concentrations measured in spring compared to autumn. Furthermore, in the period of dormancy, a process of metal elimination might appear. These aspects combined with the growth factor during the autumn leads to a diminishment of the element levels, because if the tissue was gained faster than the metal uptake occurred, the concentrations in the parasite tissue may be diluted, as described by Strong and Luoma (1981) for the free living sentinels. It can be assumed that in spring the parasite infrapopulations exibited a highly heterogeneous age structure, which was a result of the non-simultaneous maturation of the acanthocephalans. The maturation process itself is mostly affected by various factors such as host activity and physiology, water temperature and localization of acanthocephalans in the alimentary tract. For example, Dobson (1985) as well as Bates and Kennedy (1990) reported that the place of attachment in the intestine of the definitive host also played an important role in survivorship, maturation and fecundity of acanthocephalans. Therefore it could be expected that the acanthocephalans were not able to reach the reproductive stage at the same time. The heterogeneity in age composition during the cold periods was also visible due to the missing significant differences concerning the element concentrations and mean individual weight, when compared with other seasons (see **Figure 3.1** and **Figure 3.2**).

During the summer the mean individual weight slightly increased, which indicated that almost all individuals reached the adult stage. Accordingly, the mean measured concentrations in the parasites were lower, which confirms that the accumulation process completely differed in the preadult (growth) and adult phase. While the strategy of the juvenile worms is focused on gaining both weight and growth, the adults are focused on reproduction, after which they die. It could be assumed that in the adult stage and in the reproduction phase respectively, the metabolism of *P. laevis* regarding the heavy metals and As reached the equilibrium (steady state) level, where the accumulation and elimination processes were evenly involved. The hypothesis that in spring a part of individuals have already reached the steady state

concentrations was drawn due to the missing significant differences between spring and summer, when the element concentrations were compared. On the other hand, the slightly reduced concentrations in summer might be a result of elimination through the egg release during the reproduction period. Sures *et al.* (2000*b*) reported that the acanthocephalans are able to discharge metals via the shells of their eggs. The fact was proven by the higher Pb concentrations in the eggs in comparison to the worm's body and host tissues. This kind of detoxification mechanism probably appears not only in the archiacanthocephalan *Moniliformis moniliforms*, for which it was firstly described. Our study suggests the same for the group of paleacanthocephalans, to which *P. laevis* belongs to. It seems that the process comprises all accumulated by the parasite elements, not only lead, which was obvious from the similarity of the concentration pattern throughout the year.

Thus, from the obtained data was designed a model, which represents the accumulation kinetic of heavy metals and As under natural conditions (**Figure 3.4**). The model comprises the suggested by Sures (2008*b*) uptake progress, considering barbel's specific biology regarding the local climate conditions. The slightly blurred picture in comparison to laboratory studies persists due to the possibility of the barbel to obtain infection throughout all warm months. This reduces on the other hand the homogeneity of the acanthocephalan infrapopulations – the individuals are not in the same development stage and respectively the exposure duration is different for each individual. Under laboratory conditions the infection as well as the exposure are launched simultaneously, which is actually not the case in the nature. Therefore, there is a shift in accumulation process when the initial concentrations are compared. The suggested model was fitted over the year and covered approximately the life spawn of *P. laevis*, which probably envelops 7-8 months according to the element concentration data. The model comprises also the essential metals like Cu and Zn. Their concentrations showed seasonal pattern similar to the toxic elements As, Cd and Pb, although the essential metals are known to be regulated by the host's metabolism (Merian, 2004).

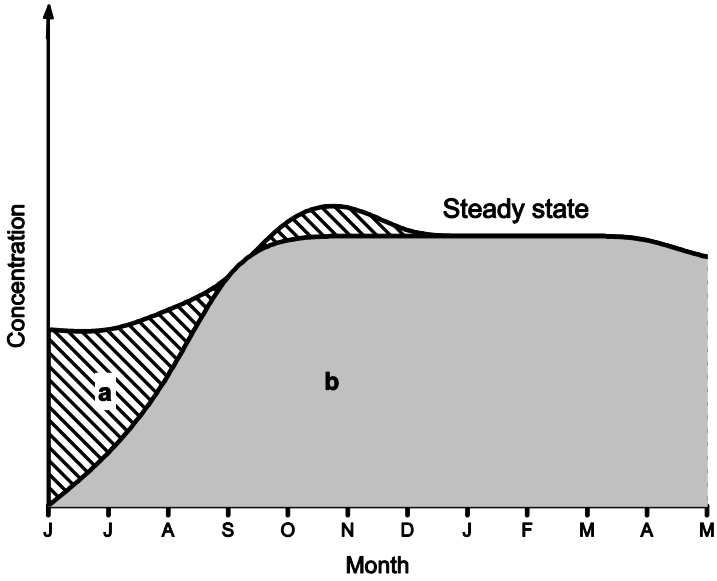

Figure 3.4. Model of metal accumulation by *P. laevis* derived from data obtained from the thesis (a) and uptake kinetic suggested by Sures (2008*b*) (b).

As summarized by Luoma and Rainbow (2008), it is necessary to study the seasonal effects on metal concentration in a particular monitor organism in order to make the impact surveys more precise after ascertaining the best period for sampling. As previously discussed, seasonal deviations of metal uptake exist even in already established sentinels such as bivalves and are mostly associated with the reproductive cycle and growth (Luoma and Rainbow, 2008). For instance, after the release of gametes, the bivalves enter a period of utilization of energy reserves characterized by accelerated metabolism and metal uptake, respectively. Moreover, the accumulation process runs differently according to the size of the mussels, whereas the smaller one showed a higher accumulation activity than bigger specimens as demonstrated by Wang and Fisher (1997). When drawing parallels between free living sentinels and parasites it seems that fish acanthocephalans show some advantages if they are taken as metal monitors. Even the fact that the fish are able to obtain randomly infection during the active periods and thus to increase the heterogeneity of age composition of their acanthocephalan infrapopulations, make the parasites a more flexible tool for assessment of metal pollution, because the concentrations in the bigger part of their lifespan remain similar (for instance in spring and summer months). For that reason, there is no need the sampling periods to be exactly considered like for the other sentinels. However, for the realization of long terms studies it would be useful and more representative if the sampling activities occurred in the same season, otherwise the life cycle should be taken into account.

4 Application of acanthocephalan *Pomphorhynchus laevis* from its host barbel (*Barbus barbus*) as metal indicator in the Danube River

4.1 Introduction

The permanent contamination of aquatic habitats caused by human activities has become one of the major problems in the era of global industrialization and urbanization. However, the anthropogenic impact is not only expressed in the form of organic pollution (euthrophication), but contamination with several toxic substances (e.g. heavy metals, PCBs) might also affect the functionality and integrity of aquatic ecosystems. Mostly, chemical pollution and in particular contamination with heavy metals is considered to have an anthropogenic source. However, natural geogenic deposition might be an important factor for heavy metal pollution as well. Thus, the detection and management of heavy metal loads in the aquatic environment is very important from the ecological point of view. Moreover, various aquatic organisms are used for human consumption and for this reason knowledge about metal contamination is extremely important from the public health view point.

The large river systems are mostly affected by anthropogenic activities, due to their large drainage area. After the Volga River, the Danube River is the second largest river in Europe with a total length of 2850 km. Its entire catchment area covers about 801,463 km^2 and is shared by ten European countries, which have agreed to monitor the environmental quality of the river and therefore launched Joint Danube Surveys (JDS) and other monitoring programs, to produce comparable and reliable information on water quality and pollution (JDS 2001, 2007; TNMN, 1996).

Recently, various sentinel organisms such as bivalves (Arndt *et al*. 1987; Reeders *et al*. 1993; Dallinger, 1994; Gunkel, 1994) have been implemented for assessing levels of pollution in aquatic habitats. However, in the last two decades fish parasites attained increasing attention, as they appear to be a more precise tool for detecting metal loads in aquatic habitats. Due to their enormous accumulation capacity, especially for some elements with severe toxic effects on biota (e.g. cadmium, lead), fish acanthocephalans are good candidates as bioaccumulation indicators. In a number of studies concentrations of metals were reported to be 10^2 to 10^5 times higher in the parasite than in the water column and the sediment (Sures *et al*. 1994*a*; Schludermann *et al*. 2003; Thielen *et al*. 2004). Comparative studies between fish acanthocephalans and established sentinels such as the zebra mussel (*Dreissena polymorpha*) demonstrated the advantage of using parasites, as their accumulation capacity highly exceeds

that of other accumulation indicators (Sures *et al*. 1999*b*). Thus, it can be concluded that fish acanthocephalans are applicable as a sensitive metal indicator for environmental monitoring procedures (Vidal-Martinez *et al*. 2010).

In order to use endoparasites as bioindicators some requirements have to be met, as suggested by Kennedy (1997): most importantly, the fish host should be abundant and easy to be sampled and secondly, the parasites must be highly abundant and prevalent among the host population. The fresh water cyprinid *Barbus barbus* and its parasite *Pomphorhynchus laevis* seems to be a promising model for metal monitoring as they fulfil the above mentioned requirements. The barbel is the second largest native cyprinid species in Europe, is wide spread in the epipotamal of large rivers such as the Danube River and is known to show high infection levels with the intestinal acanthocephalan *P. laevis* (Kakacheva-Avramova, 1962, 1977; Margaritov, 1959, 1966; Moravec *et. al.* 1997; Schludermann *et al*. 2003; Thielen *et al*. 2004; Laimgruber *et al*. 2005; Nachev and Sures, 2009). This parasite species is already well investigated in terms of its metal accumulation (summarized by Sures, 2003; 2004*b*).

The aim of this study was to perform a long term metal monitoring (in the period from summer 2004 to summer 2007) and to analyse longitudinal patterns of metal distribution in the Danube using the acanthocephalan *P. laevis*. Infected barbels were collected from different sampling sites along the lower Danube in Bulgaria. Additionally, some fish samples delivered by the second Joint Danube Survey (JDS2) conducted in summer 2007, were also analysed in order to detect differences between the upper and the lower Danube reaches. The results were compared and correlated with the available metal monitoring data provided by International Commission for Protection of Danube River (ICPDR) for water and suspended particulate matter (SPM) in order to obtain all possible information on the presence and bioavailability of trace metals in the Danube River.

4.2 Materials and Methods

4.2.1 Fish samples

The study area was mainly restricted to the Bulgarian part of the Danube River. In the study period from summer 2004 to summer 2007 up to 35 barbels per sampling were collected in a seasonal manner (April, July and October) from two localities. To represent the upper Danube reaches on the Bulgarian river site one sampling site was situated at river kilometre 834 near the town Vidin. Furthermore, it was in a distance of about 10-15 km downstream from the inflow of the river Timok, which is known as a one of the major sources for heavy metal

pollution in this part of the Danube (Literathy *et al*. 2002; 2009). The second sampling site was near the town Kozloduy (685 km) - about 150 river kilometres downstream from Vidin. Additionally, from spring 2006 to summer 2007, barbels were collected from a site near the town Silistra (375 km), which represents the last Bulgarian locality in eastward direction of the river (for details see **Chapter 1**; **Figure 1.1**). In summer 2007, during the second Joint Danube Survey (JDS 2), fish samples were obtained also from four localities in the upper Danube reach (**Table 4.1**).

Table 4.1. JDS2 (second Joint Danube Survey) sampling site description.

Sampling site code	River kilometre	Location	Number of barbels
JDS 13	1930	Vienna downstream	n=3
JDS 16	1869	Bratislava upstream	n=10
JDS 26	1707	Szob	n=4
JDS 32	1648	Budapest downstram	n=3

In order to evaluate the use of parasites as accumulation indicators, a long term monitoring during 4 years, a longitudinal profile in the Bulgarian section of the Danube River and a longitudinal profile of the entire Danube basin were conducted. All barbels taken for long term metal monitoring were caught in the same season (e.g. summer) at one sampling site during the entire period of investigation in order to detect changes of metal concentration in the lower Danube. Therefore, eight medium sized fishes per summer were selected during the period 2004-2007 from Kozloduy. Comparative studies for all three sampling sites in Bulgaria were performed during summer 2006 - eight barbels were taken from each locality. The JDS2 fish samples collected during the survey in 2007 (**Table 4.1**) were compared with our barbels collected in summer 2007 at site Kozloduy.

4.2.2 Heavy metal analysis

The concentrations of arsenic (As), cadmium (Cd), colbalt, (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), vanadium (V) and zinc (Zn) were were analyzed in fish tissue (muscle, intestine and liver) and parasite samples using inductively coupled plasma mass spectrometry (ICP-MS) (for details see **Chapter 2**).

The accuracy of the analytical procedure was verified with the help of a standard reference material (DORM-3, National Research Council, Canada) of dogfish (*Squalus acanthias*). After the analysis, the accuracy rates of seven certified elements were checked. Their values

ranged between 87% and 106%, whereas the highest accuracy was obtained for iron (100%) (**Chapter 2**; **Table 2.1**).

4.2.3 Data analyses and statistical treatment

The Mann-Whitney *U*-test was applied to test for significant differences between sampling sites as well as between metal concentrations in the host-parasite system with data published for the river Danube. In order to express the accumulation capacity of fish acanthocephalans, the mean bioconcentration factors were calculated according to Sures *et al*. (1999*a*) as follows: ($C_{[P.laevis]}$ / $C_{[host\ tissue]}$). Bioconcentration factors were also determined with respect to concentrations in water - $C_{[P.laevis]}$ / $C_{[water]}$. In order to test for significant differences of metal concentrations between host tissues and parasites, the Wilcoxon matched pairs test was applied.

4.2.4 Background metal monitoring data

Data published by the ICPDR was used to correlate the metal concentrations in the barbel - *P. laevis* system with those in different matrices of the Danube. The data used for the present study were from the Trans Nation Monitoring Network (**Table 4.2**; TNMN, 2009) program for the period 2003-2006 (monthly metal monitoring in water column) as well as from both Danube expeditions – Joint Danube Surveys (JDS1 in 2001 and JDS2 in 2007), which delivered an overview of concentrations in water and suspended particulate matter (SPM) (Literathy *et al*. 2002; 2009). The metal levels close to our fish sampling sites were used for comparisons with the parasite data. The JDS sampling sites for the Bulgarian river stretch were River Timok (confluence with the Danube at km 845 – approximately 10 km upstream from our first sampling site Vidin), Rusenski Lom (450 km, 75 river kilometre upstream from Silistra) and Silistra (375 km; labelled by JDS2 as Upstream Arges). The TNMN monitoring sites were, respectively, Novo Selo (833 km), approximately 5 river km upstream form the sampling site Vidin, Iskar (641 km) about 40 river km downstream from Kozloduy and a monitoring point directly in Silistra (375 km) (see **Table 4.2**). Data from the Danube Surveys was used for comparing the longitudinal distribution of heavy metals in the Danube River basin.

Table 4.2. Data on aqueous element concentrations according to TNMN (2009) for upper and lower sites of the Bulgarian part of Danube River.

Element	Year	Novo Selo (833km) (5 km upstream of Vidin)	Iskar (675 km) (40 km downstream of Kozloduy)	Silistra (375 km)
		Right bank	Righ bank	Right bank
As	2003	3.075	3.345	2.813
	2004	3.4	3.667	n/a
	2005	2.2	2.692	n/a
	2006	2.275	2.382	0.3239
Cd	2003	0	0	1
	2004	0	1.167	0
	2005	0	1.825	0
	2006	0	0	0
Cu	2003	14.9	9.083	6
	2004	18.67	6.417	2.545
	2005	17.5	5.158	0
	2006	23.5	6.1	0
Mn	2003	0.0178	0.0167	0.0303
	2004	0.0141	0.0108	0.0327
	2005	0.0143	0.0045	0.0519
	2006	0.0198	0.0091	0.0373
Ni	2003	1.975	2.778	2
	2004	2.417	0	2.727
	2005	4.917	0	0
	2006	9	0	0
Pb	2003	1.842	2.333	2.75
	2004	2	2.583	0
	2005	1.833	2.767	0
	2006	0	2.364	0
Zn	2003	23.42	43.83	27.08
	2004	24.83	24.67	23.27
	2005	22	29.73	21.5
	2006	20.92	20	19.83

n/a: Data not available

4.3 Results

4.3.1 Element concentrations in the host-parasite system

Similar to the results presented in the **Chapters 2** and **Chapter 3**, the acanthocephalan *P. laevis* showed significantly higher concentrations of the elements As, Cd, Cu, Zn and Pb than the host tissues (**Table 4.3**; **Table 4.4**; **Table 4.5**). Generally, *P. laevis* showed the highest accumulation capacity in comparison with fish organs for Pb, followed by Cd, Cu, Zn and As (see **Table 4.6**). With the exception of As, the concentration of these elements also exceeded the concentrations of the local aqueous environment. This was additionally proved by the bioconcentration factors for the water (**Table 4.6**).

4.3.2 Longitudinal profile of element concentrations in the Bulgarian part of the Danube River in 2006

The metal monitoring conducted in summer 2006 at the three selected sampling sites in Bulgaria showed that the concentrations of the elements As and Cd decreased in downstream direction in the parasites (**Table 4.3** and **Figure 4.1**). The essential elements Cu and Zn were found in lowest mean concentrations in Kozloduy, whereas the levels in Vidin were 2 times higher. In contrast, Pb was found in similar concentrations in the parasites at all sampling sites, with levels ranging between 5.19 µg/kg for Kozloduy and 5.93 µg/kg for Silistra. The distribution of some other elements such as manganese (Mn) in *P. laevis*, revealed a similar pattern as the elements As and Cd. Its concentration decreased in the lower part of the Bulgarian Danube stretch with significantly lower concentrations in Silistra than in Vidin. Manganese was also found in higher quantities in the parasites compared to fish tissues although *P. laevis* had lower levels than the water as can be seen from the mean bioconcentration factors (**Table 4.6**).

Generally, no clear longitudinal trend was detected for the element concentrations in fish tissues, as the data showed a high heterogeneity. One exception was Ni, whose mean concentration in muscle was elevated in upper section of the Danube- Vidin (2.06 mg/kg); Kozloduy (0.66 mg/kg); Silistra (0.88 mg/kg) (see **Table 4.3**).

Table 4.3. Element concentrations in *P. laevis* and different host tissues obtained in summer 2006 for the Bulgarian part of Danube River.

Sampling site		Vidin	Kozloduy	Silistra
As	Muscle	0.07 (±0.04)	0.21 (±0.15)	0.08 (±0.05)
	Intestine	0.15 (±0.10)	0.35 (±0.20)	0.09 (±0.07)
	Liver	0.16 (±0.07)	0.54 (±0.49)	0.16 (±0.13)
	P. laevis	1.53 (±0.97)	1.01 (±0.60)	0.91 (±0.86)
Cd	Muscle	0.01 (±0.01)	0.01 (±0.01)	0.01 (±0.01)
	Intestine	0.08 (±0.05)	0.11 (±0.07)	0.03 (±0.01)
	Liver	0.10 (±0.05)	0.08 (±0.04)	0.04 (±0.02)
	P. laevis	1.89 (±1.23)	1.34 (±0.55)	1.21 (±0.65)
Co	Muscle	0.02 (±0.01)	0.02 (±0.01)	0.02 (±0.01)
	Intestine	0.11 (±0.17)	0.17 (±0.14)	0.05 (±0.04)
	Liver	0.04 (±0.02)	0.04 (±0.01)	0.03 (±0.01)
	P. laevis	0.10 (±0.11)	0.10 (±0.07)	0.05 (±0.01)
Cu	Muscle	1.14 (±0.75)	1.57 (±1.29)	0.76 (±0.21)
	Intestine	2.90 (±0.99)	4.79 (±2.44)	2.50 (±0.68)
	Liver	12.09 (±6.10)	13.40 (±8.11)	13.10 (±12.34)
	P. laevis	57.95 (±40.28)	53.13 (±24.13)	96.01 (±47.23)
Fe	Muscle	20.18 (±10.92)	10.21 (±4.72)	8.36 (±3.05)
	Intestine	68.85 (±66.84)	70.92 (±17.87)	38.56 (±15.23)
	Liver	92.96 (±22.06)	81.34 (±39.42)	65.89 (±20.72)
	P. laevis	36.22 (±20.84)	41.27 (±23.28)	30.21 (±8.79)
Mn	Muscle	0.56 (±0.21)	0.35 (±0.11)	0.31 (±0.13)
	Intestine	7.38 (±9.91)	7.35 (±6.37)	2.28 (±1.32)
	Liver	1.79 (±1.19)	1.49 (±0.77)	1.10 (±0.29)
	P. laevis	10.55 (±10.41)	7.64 (±5.87)	5.13 (±1.26)
Mo	Muscle	n.d.	n.d.	0.02 (±0.01)
	Intestine	0.05 (±0.01)	0.06 (±0.02)	0.07 (±0.05)
	Liver	0.18 (±0.08)	0.28 (±0.16)	0.26 (±0.19)
	P. laevis	0.03 (±0.02)	0.10 (±0.06)	0.11 (±0.13)
Ni	Muscle	2.06 (±1.34)	0.66 (±0.61)	0.88 (±0.97)
	Intestine	1.45 (±0.92)	2.28 (±1.16)	2.05 (±1.19)
	Liver	0.48 (±0.36)	0.21 (±0.15)	0.72 (±0.62)
	P. laevis	0.37 (±0.27)	0.55 (±0.28)	0.17 (±0.14)
Pb	Muscle	0.01 (±0.01)	0.004 (±0.003)	0.01 (±0.01)
	Intestine	0.09 (±0.07)	0.12 (±0.10)	0.09 (±0.11)
	Liver	0.05 (±0.04)	0.03 (±0.02)	0.02 (±0.01)
	P. laevis	5.70 (±4.77)	5.19 (±3.74)	5.93 (± 3.66)
V	Muscle	0.04 (±0.01)	0.04 (±0.01)	0.03 (±0.01)
	Intestine	0.11 (±0.11)	0.29 (±0.36)	0.09 (±0.04)
	Liver	0.25 (±0.14)	0.17 (±0.16)	0.14 (±0.10)
	P. laevis	0.07 (±0.02)	0.10 (±0.04)	0.05 (±0.01)
Zn	Muscle	4.14 (±0.75)	4.76 (±1.51)	4.85 (±1.82)
	Intestine	12.69 (±1.77)	10.40 (±1.16)	12.88 (±4.41)
	Liver	18.31 (±4.13)	19.23 (±5.84)	16.12 (±5.14)
	P. laevis	52.34 (±36.22)	34.83 (±16.66)	109.38 (±62.10)

n.d.: concentrations below detection limit

Table 4.4. Element concentrations in *P. laevis* and different host tissues obtained in summer 2007 for upper and lower Danube.

Element		Danube sampling sites				
		JDS 13	JDS 16	JDS 26	JDS 32	Kozloduy
As	Muscle	0.13 (± 0.17)	n.d.	n.d.	n.d.	0.07 (±0.03)
	Intestine	0.46 (± 0.19)	0.26 (± 0.25)	n.d.	n.d.	0.21 (±0.13)
	Liver	0.77 (± 0.17)	0.30 (± 0.30)	0.33 (± 0.30)	0.16 (± 0.22)	0.24 (±0.13)
	P. laevis	1.17 (± 0.11)	0.83 (± 0.45)	0.22 (± 0.13)	0.37 (± 0.27)	0.85 (±0.64)
Cd	Muscle	0.01 (± 0.01)	0.004 (± 0.003)	0.005 (± 0.002)	0.002 (± 0.001)	0.02 (±0.02)
	Intestine	0.02 (± 0.01)	0.04 (± 0.05)	0.03 (± 0.02)	0.01 (± 0.01)	0.19 (±0.14)
	Liver	0.02	0. 08 (± 0.08)	0.14 (± 0.12)	0.08 (± 0.06)	0.21 (±0.18)
	P. laevis	0.14 (± 0.05)	0.30 (± 0.34)	0.30 (± 0.23)	0. 11 (± 0.04)	1.82 (±1.36)
Co	Muscle	0.02 (± 0.006)	0.01 (± 0.01)	0.01 (± 0.002)	0.003 (± 0.002)	0.01 (±0.01)
	Intestine	0.18 (± 0.06)	0.20 (± 0.22)	0.03 (± 0.03)	0.07 (± 0.05)	0.11 (±0.05)
	Liver	0.12 (± 0.01)	0.08 (± 0.06)	0.03 (± 0.004)	0.04 (± 0.02)	0.04 (±0.01)
	P. laevis	0.25 (± 0.10)	0.17 (± 0.11)	0.05 (± 0.02)	0.04 (± 0.03)	0.07 (±0.04)
Cu	Muscle	0. 62 (± 0.17)	0.81 (± 0.27)	0.78 (± 0.11)	0.54 (± 0.13)	0.78 (±0.40)
	Intestine	3.09 (± 0.22)	2.62 (± 1.02)	2.03 (± 0.89)	1.70 (± 0.12)	4.43 (±2.01)
	Liver	11.5 (± 1.33)	10.3 (± 6.56)	13.2 (± 7.55)	12.7 (± 2.92)	11.65 (±6.28)
	P. laevis	22.9 (± 4.53)	32.6 (± 24.0)	16.6 (± 12.7)	8.85 (± 5.11)	26.06 (±15.27)
Fe	Muscle	10.4 (± 1.20)	13.6 (± 8.85)	11.7 (± 2.63)	11.9 (± 4.19)	16.36 (±8.43)
	Intestine	60.0 (± 25.3)	49.3 (± 19.9)	27.7 (± 14.2)	52.6 (± 28.2)	67.71 (±41.22)
	Liver	92.1 (± 32.6)	80.4 (± 23.7)	73.1 (± 5.02)	89.3 (± 40.8)	99.99 (±45.51)
	P. laevis	58.0 (± 30.4)	36.6 (± 11.1)	26.7 (± 15.1)	76.7 (± 47.6)	31.11 (±16.20)
Mn	Muscle	0.46 (± 0.12)	0.28 (± 0.10)	0.22 (± 0.06)	0.20 (± 0.07)	0.49 (±0.46)
	Intestine	7.88 (± 4.34)	6.30 (± 6.43)	3.84 (± 2.58)	3.67 (± 2.82)	4.36 (±3.63)
	Liver	3.96 (± 0.17)	1.80 (± 1.20)	1.06 (± 0.30)	1.33 (± 0.52)	1.56 (±1.00)
	P. laevis	7.90 (± 1.84)	9.37 (± 5.38)	4.85 (± 2.33)	6.53 (± 3.86)	6.54 (±2.46)
Mo	Muscle	0.22 (± 0.02)	0.01 (± 0.01)	n.d.	n.d.	n.d.
	Intestine	0.08 (± 0.03)	0.04 (± 0.01)	0.05 (± 0.01)	0.05 (± 0.02)	0.06 (±0.02)
	Liver	0.15 (± 0.01)	0.21 (± 0.06)	0.15 (± 0.01)	0.23 (± 0.11)	0.25 (±0.04)
	P. laevis	0.04 (± 0.03)	0.02 (± 0.01)	0.05 (± 0.04)	0.02 (± 0.01)	0.06 (±0.03)
Ni	Muscle	0.38 (± 0.24)	0.78 (± 1.12)	0.71 (± 0.57)	0.91 (± 0.79)	0.55 (±0.35)
	Intestine	3.25 (± 1.17)	1.01 (± 0.93)	1.16 (± 0.49)	1.93 (± 0.83)	2.07 (±1.24)
	Liver	2.65 (± 2.43)	0.77 (± 1.46)	0.44 (± 0.53)	0.74 (± 0.89)	0.49 (±0.26)
	P. laevis	0.27 (± 0.07)	0.61 (± 0.78)	0.26 (± 0.19)	0.25 (± 0.22)	0.35 (±0.29)
Pb	Muscle	0.01 (± 0.003)	0.02 (± 0.02)	0.004 (± 0.003)	0.003 (± 0.001)	0.01 (±0.01)
	Intestine	0.18 (± 0.12)	0.13 (± 0.14)	0.04 (± 0.02)	0.07 (± 0.06)	0.17 (±0.16)
	Liver	0.06 (± 0.04)	0.05 (±0.04)	0.03 (± 0.01)	0.03 (± 0.03)	0.04 (±0.03)
	P. laevis	1.75 (± 1.34)	2.52 (± 1.75)	2.67 (± 3.45)	0.82 (± 0.69)	3.02 (±1.71)
V	Muscle	0.02 (± 0.001)	0.02 (± 0.01)	0.03 (± 0.01)	0.03 (± 0.003)	0.04 (±0.02)
	Intestine	0.25 (± 0.16)	0.23 (± 0.23)	0.08 (± 0.05)	0.18 (± 0.13)	0.18 (±0.12)
	Liver	0.12 (± 0.02)	0.40 (± 0.31)	0.69 (± 0.67)	1.21 (± 1.37)	0.32 (±0.33)
	P. laevis	0.06 (± 0.01)	0.06 (± 0.02)	0.03 (± 0.01)	0.06 (± 0.02)	0.08 (±0.05)
Zn	Muscle	3.64 (± 0.66)	3.56 (± 0.50)	3.45 (± 0.21)	2.93 (± 0.25)	4.11 (±1.01)
	Intestine	13.7 (± 0.77)	12.7 (± 6.25)	12.3 (± 1.27)	12.0 (± 0.71)	13.61 (±5.64)
	Liver	19.8 (± 4.07)	21.9 (± 7.83)	18.4 (± 3.72)	22.4 (± 3.87)	20.34 (±5.02)
	P. laevis	20.9 (± 5.28)	28.8 (± 8.95)	83.6 (± 118.6)	39.8 (± 14.6)	46.45 (±14.83)

n.d.: concentrations below detection limit

Table 4.5. Element concentrations in *P. laevis* and in different host tissues measured for the period summer 2004 - summer 2007 at site Kozloduy.

Year		Summer 2004	Summer 2005	Summer 2006	Summer 2007
As	Muscle	0.08 (±0.04)	0.16 (±0.09)	0.21 (±0.15)	0.07 (±0.03)
	Intestine	0.37 (±0.23)	0.27 (±0.23)	0.35 (±0.20)	0.21 (±0.13)
	Liver	0.20 (±0.09)	0.45 (±0.36)	0.54 (±0.49)	0.24 (±0.13)
	P. laevis	0.60 (±0.24)	0.93 (±0.49)	1.01 (±0.60)	0.85 (±0.64)
Cd	Muscle	0.01 (±0.01)	0.02 (±0.01)	0.01 (±0.01)	0.02 (±0.02)
	Intestine	0.28 (±0.21)	0.10 (±0.04)	0.11 (±0.07)	0.19 (±0.14)
	Liver	0.17 (±0.08)	0.11 (±0.08)	0.08 (±0.04)	0.21 (±0.18)
	P. laevis	3.92 (±2.87)	1.92 (±1.09)	1.34 (±0.55)	1.82 (±1.36)
Co	Muscle	0.01 (±0.01)	0.02 (±0.01)	0.02 (±0.01)	0.01 (±0.01)
	Intestine	0.11 (±0.08)	0.09 (±0.10)	0.17 (±0.14)	0.11 (±0.05)
	Liver	0.06 (±0.04)	0.04 (±0.02)	0.04 (±0.01)	0.04 (±0.01)
	P. laevis	0.07 (±0.03)	0.08 (±0.08)	0.10 (±0.07)	0.07 (±0.04)
Cu	Muscle	0.58 (±0.32)	0.92 (±0.32)	1.57 (±1.29)	0.78 (±0.40)
	Intestine	7.85 (±2.56)	4.26 (±3.18)	4.79 (±2.44)	4.43 (±2.01)
	Liver	8.22 (±2.83)	6.99 (±2.46)	13.40 (±8.11)	11.65 (±6.28)
	P. laevis	84.63 (±42.38)	56.71 (±24.75)	53.13 (±24.13)	26.06 (±15.27)
Fe	Muscle	15.56 (±8.06)	9.17 (±3.11)	10.21 (±4.72)	16.36 (±8.43)
	Intestine	97.56 (±33.99)	77.55 (±63.73)	70.92 (±17.87)	67.71 (±41.22)
	Liver	205.10 (±79.11)	71.18 (±35.34)	81.34 (±39.42)	99.99 (±45.51)
	P. laevis	39.54 (±14.16)	57.48 (±25.71)	41.27 (±23.28)	31.11 (±16.20)
Mn	Muscle	0.45 (±0.34)	0.41 (±0.12)	0.35 (±0.11)	0.49 (±0.46)
	Intestine	5.55 (±5.94)	7.79 (±9.56)	7.35 (±6.37)	4.36 (±3.63)
	Liver	2.07 (±1.20)	1.64 (±0.94)	1.49 (±0.77)	1.56 (±1.00)
	P. laevis	5.76 (±1.82)	6.38 (±4.56)	7.64 (±5.87)	6.54 (±2.46)
Mo	Muscle	n.d.	n.d.	n.d.	n.d.
	Intestine	0.11 (±0.04)	0.04 (±0.01)	0.06 (±0.02)	0.06 (±0.02)
	Liver	0.09 (±0.04)	0.12 (±0.07)	0.28 (±0.16)	0.25 (±0.04)
	P. laevis	0.04 (±0.02)	0.05 (±0.03)	0.10 (±0.06)	0.06 (±0.03)
Ni	Muscle	0.14 (±0.23)	0.50 (±0.33)	0.66 (±0.61)	0.55 (±0.35)
	Intestine	3.99 (±2.12)	1.25 (±0.85)	2.28 (±1.16)	2.07 (±1.24)
	Liver	0.95 (±0.89)	0.43 (±0.23)	0.21 (±0.15)	0.49 (±0.26)
	P. laevis	0.34 (±0.20)	0.51 (±0.30)	0.55 (±0.28)	0.35 (±0.29)
Pb	Muscle	0.02 (±0.01)	0.004 (±0.004)	0.004 (±0.003)	0.01 (±0.01)
	Intestine	0.16 (±0.11)	0.18 (±0.18)	0.12 (±0.10)	0.17 (±0.16)
	Liver	0.07 (±0.03)	0.03 (±0.02)	0.03 (±0.02)	0.04 (±0.03)
	P. laevis	7.32 (±2.98)	6.04 (±4.04)	5.19 (±3.74)	3.02 (±1.71)
V	Muscle	0.03 (±0.01)	0.04 (±0.01)	0.04 (±0.01)	0.04 (±0.02)
	Intestine	0.21 (±0.16)	0.23 (±0.22)	0.29 (±0.36)	0.18 (±0.12)
	Liver	0.15 (±0.07)	0.27 (±0.48)	0.17 (±0.16)	0.32 (±0.33)
	P. laevis	0.08 (±0.03)	0.10 (±0.08)	0.10 (±0.04)	0.08 (±0.05)
Zn	Muscle	4.42 (±1.47)	4.21 (±0.79)	4.76 (±1.51)	4.11 (±1.01)
	Intestine	25.09 (±5.05)	13.12 (±2.49)	10.40 (±1.16)	13.61 (±5.64)
	Liver	26.89 (±11.94)	16.14 (±6.68)	19.23 (±5.84)	20.34 (±5.02)
	P. laevis	100.78 (±63.42)	75.42 (±57.03)	34.83 (±16.66)	55.47 (±33.13)

n.d.: concentrations below detection limit

Table 4.6. Bioconcentration factors calculated for summer 2006 at three sampling sites in Bulgaria.

		Vidin	Kozloduy	Silistra
As	Muscle	20.53	4.84	11.94
	Intestine	9.91	2.86	10.07
	Liver	9.33	1.87	5.73
	Water	0.67	0.42	2.81
Cd	Muscle	146.31	132.72	175.68
	Intestine	22.70	11.68	38.87
	Liver	19.01	16.04	27.50
	Water	1.89	1.34	1.21
Cu	Muscle	50.93	33.85	126.57
	Intestine	20.01	11.10	38.35
	Liver	4.79	3.96	7.33
	Water	2.47	8.71	96.01
Fe	Muscle	1.80	4.04	3.61
	Intestine	0.53	0.58	0.78
	Liver	0.39	0.51	0.46
	Water	0.14	0.47	0.05
Mn	Muscle	18.74	21.88	16.34
	Intestine	1.43	1.04	2.25
	Liver	5.90	5.14	4.66
	Water	0.53	0.84	0.14
Ni	Muscle	0.18	0.83	0.19
	Intestine	0.26	0.24	0.08
	Liver	0.78	2.59	0.23
	Water	0.04	0.55	0.17
Pb	Muscle	452.03	1250.01	737.83
	Intestine	60.56	42.16	67.50
	Liver	107.80	151.08	271.68
	Water	5.70	2.19	5.93
Zn	Muscle	12.65	7.31	22.57
	Intestine	4.13	3.35	8.49
	Liver	2.86	1.81	6.78
	Water	2.50	1.74	5.52

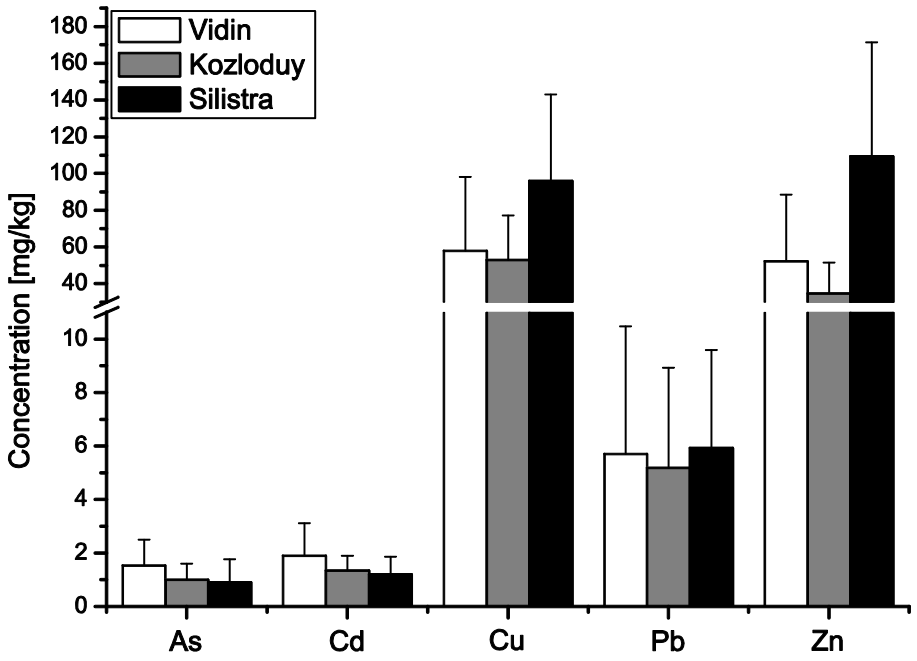

Figure 4.1. Longitudinal profile of elements accumulated by *P. laevis* obtained for summer 2006 in Bulgarian part of Danube River.

4.3.3 Longitudinal profile of element concentrations in the Danube River in 2007

Four localities situated in the upper Danube were compared with Kozloduy in the lower Danube in order to obtain a longitudinal metal profile. According to metal concentrations in the parasites there were clear differences between the upper and lower Danube for the toxic elements Cd and Pb (**Figure 4.2** and **Table 4.4**). Increased mean values for their concentrations were detected in the lower part of the river – they were up to 16 and 4 times higher for Cd and Pb, respectively. Concentrations of the other elements in *P. laevis* such as As and Cu showed increased mean values at the upper two localities (downstream from Vienna; upstream from Bratislava) as well as in the lower Danube in Bulgaria (Kozloduy, see **Table 4.4**). The levels of Zn, on the other hand, increased also in downstream direction, although insufficient number of barbels were investigated from sampling sites Szob (site JDS 26) and downstream from Budapest (site JDS 32) (**Table 4.4**).

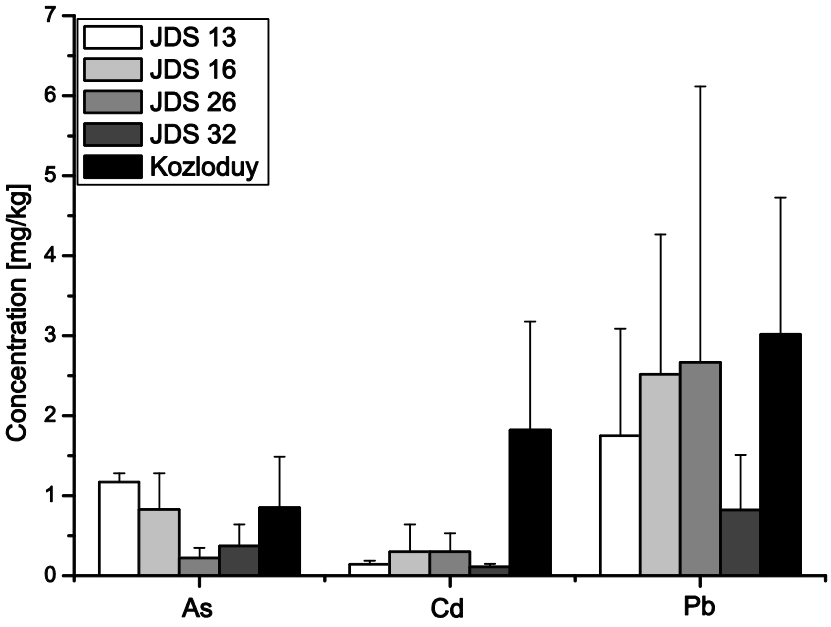

Figure 4.2. Danube's longitudinal profile of elements As, Cd and Pb in *P. laevis*, obtained in summer 2007.

4.3.4 Long term monitoring of element concentrations in the lower Danube

Elements concentrations in the parasites (Cd, Cu, Pb, Zn) showed a decreasing tendency from summer 2004 to summer 2007 (**Table 4.5** and **Figure 4.3**) at Kozloduy indicating an improvement of the water quality. Fluctuations in parasite concentrations were observed only for the element As, with increasing mean concentrations in 2005 and 2006 and decreasing levels in 2007, thus reaching a concentration similar to the one measured in 2004 (see **Table 4.5**). In contrast to the parasites, Cd and Zn concentrations in fish tissues and particularly in fish muscle remained stable during the years. The As concentrations in barbel's muscle followed the pattern in the parasites during the period of investigation. Similar relationships were not observed for Cu and Pb. The concentrations of the other elements showed no clear tendency neither in fish organs, nor in the parasite.

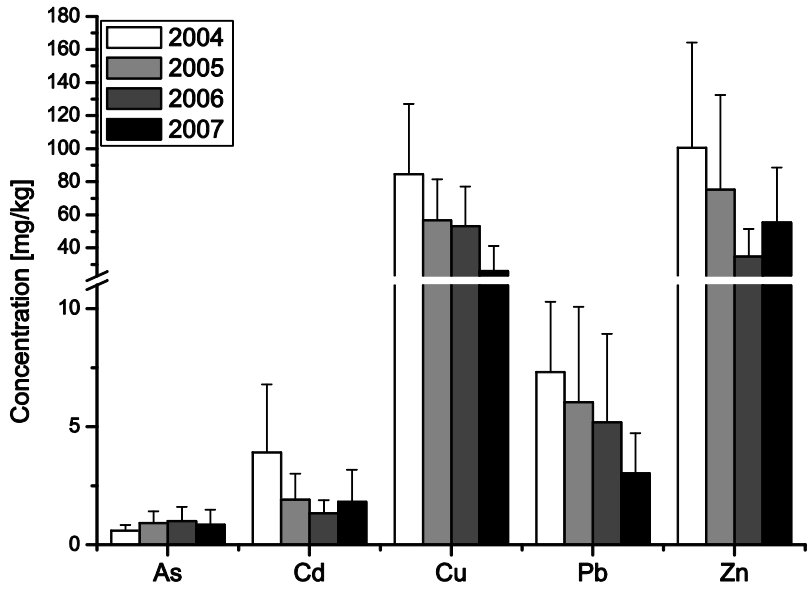

Figure 4.3. Long term monitoring of elements As, Cd, Cu, Pb and Zn in *P. laevis* at site Kozloduy (Bulgaria).

4.3.5 Comparisons between element concentrations in parasite with the available background data for water and SPM

The highest aqueous Cu concentration for the entire Danube was reported by JDS1 in 2001 and JDS2 in 2007 at the River Timok' confluence (Literathy *et al*. 2002; 2009). The impact of the tributary was also evident, at the TNMN site Novo Selo situated below the Timok effluence (see **Table 4.2**). At this site, during the JDS2 were found also extended concentrations of element Zn and Ni (Literathy *et al*. 2009). Additionally, some of the highest Cu and Zn levels were reported in the water for other tributaries such as Rusenski Lom (75 river kilometers upstream of Silistra) for Cu and Silistra (375 km) for Zn. As expected, the longitudinal profile of heavy metal concentrations in the parasites followed the pattern of Cu and Zn in the water. The reported Zn concentration at Timok confluence was 9.3 µg/L and corresponded to a mean concentration in the parasites of 52.34 µg/g at Vidin. On the other hand, the aqueous Zn concentration at site Silistra was almost 2 times higher (16.1 µg/L) compared to Timok River. Consequently, the obtained Zn levels in the acanthocephalans at site Silistra (109.38 µg/g) were in the same quantity higher as the water- about two times (chemical data from JDS2 technical report by Literathy *et al*. 2009). A similar longitudinal profile was also observed for Cu with higher concentrations in the upper and lower Danube

section in Bulgaria and lower levels in Kozloduy (Literaty *et al*. 2009).

As previously mentioned, the longitudinal profile of the other elements found in higher amounts in *P. laevis* (As, Cd, Mn and Pb), showed a decreasing tendency along the Danube in Bulgaria, except for Pb, for which concentrations in the parasite remained similar at all monitoring points (see **Table 4.3** and **Figure 4.1**). According to water concentrations available for 2006, the only significant difference was found for As when the upper and the lower reaches in the Bulgarian section of the River were compared (see **Table 4.2**): Novo Selo (2.275 µg/L); Silistra (0.3239 µg/L). Elevated aqueous concentrations were reported for Pb at locality Iskur used as a reference for our sampling site Kozloduy, since at the sites Vidin and Silistra no Pb has been detected. Cadmium, on the other hand, was below the method detection limits at all sampling sites. (see **Table 4.2**).

The comparison of metal concentrations in the parasites with levels determined for SPM during JDS2 showed identical trends (see **Figure 4.2** and **Figure 4.4**), although detailed correlation analyses could not be performed as the heavy metal raw data gathered by JDS2 are still not published.

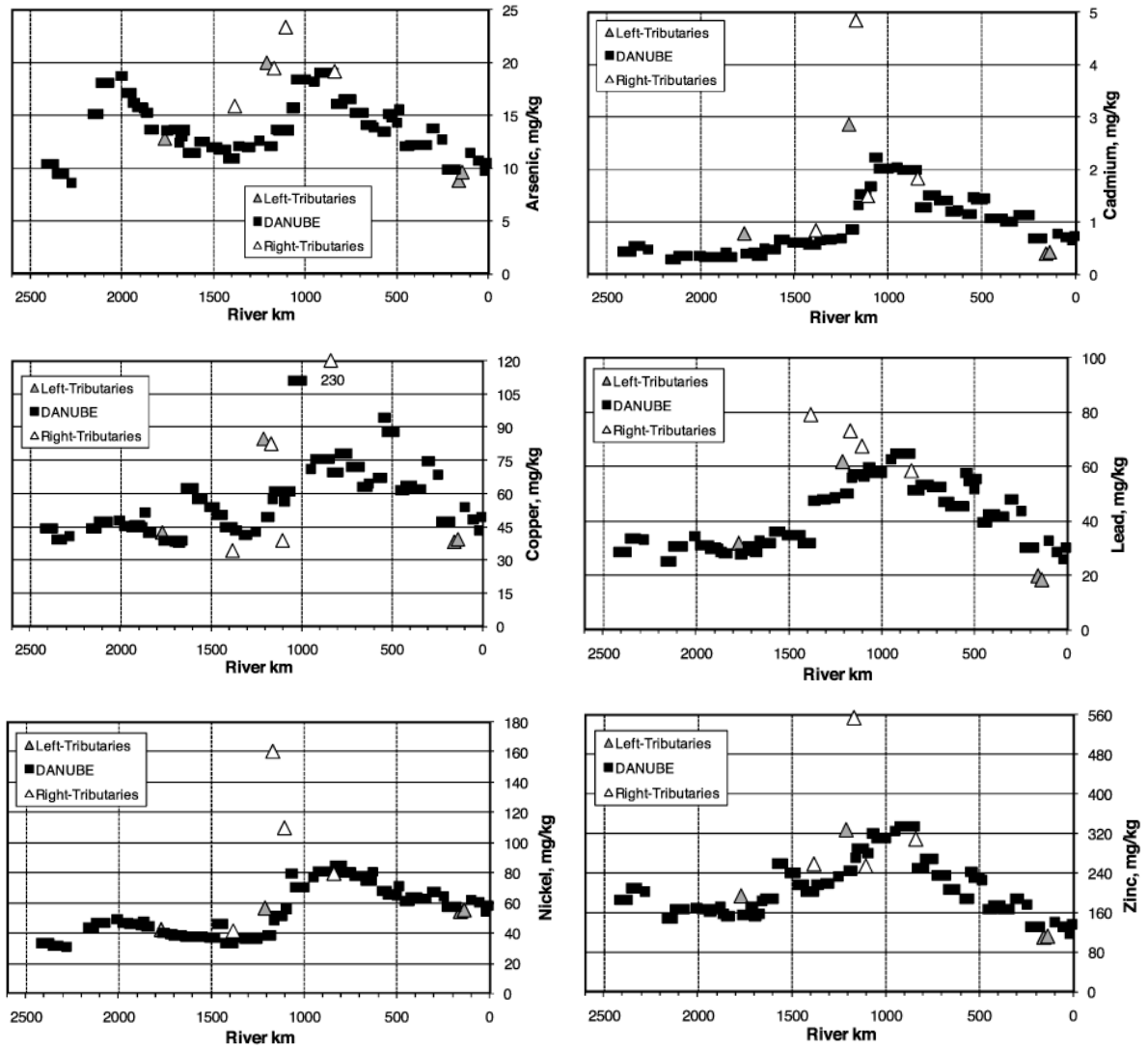

Figure 4.4. Distribution profile of As, Cd, Cu, Ni, Pb and Zn in the SPM along the Danube River during JDS2 (from Literathy *et al*. 2009).

4.4 Discussion

As demonstrated in **Chapter 2** and **Chapter 3** as well as in the present chapter, *P. laevis* showed a better accumulation capacity for the elements As, Cd, Cu, Pb and Zn than its fish host. However, As concentrations in the parasites were lower compared to those in the water, in contrast to the other elements. Therefore, it seems possible that *P. laevis* can be employed as a sentinel for the the metals Cd, Cu, Pb and Zn. These elements were also categorized by ICPDR into priority groups 1 and 2, according to their toxicological importance. Group 1 includes the elements Cd and Pb together with Hg and Ni, while the elements As, Cu and Zn were categorized in group 2 together with Bi, Co, Cr, Mo in suspended particulate matter and

Co, Ti and V in sediments. The elements Fe and Mn, for instance, were placed in group 3 as metals important for the overall assessment of water quality (Literathy *et al*. 2009). Thus, the acanthocephalans can be successfully applied as metal indicators especially for the priority metals in the Danube River basin.

The selected river stretch of the Danube in Bulgaria, where the metal monitoring survey was carried out, can be considered as an optimal part of the river for performing this kind of investigations. The lower Danube reaches suffer from the impact of some major tributaries such as Tisa and Sava (joining the Danube in Serbia at 1215 and 1170 river kilometre, respectively), which are known as the biggest pollution sources along the Danube River (Literathy *et al*. 2002; 2009). As a result of their inflow, the lower Danube reaches are polluted downstream of river km 1000. The influence of the tributaries is evident when analysing the longitudinal profiles of heavy metals and As in the SPM published by JDS2 (see **Figure 4.4;** Literathy *et al*. 2009). Furthermore, the Timok River has been polluted by copper mining and heavy metal industry in all of its drainage area since the middle of twentieth century (Antonović *et al.* 1974; Božinović *et al.* 2005). Consequently, it pollutes the Danube with various heavy metals such as cadmium, copper and lead, released by ore leaching.

The longitudinal profile of element concentrations in the parasites corresponded to the contamination profile reported for the Bulgarian river stretch (Litheraty *et al.* 2002, 2009; TNMN 2009). At the localities in upper and lower part of the river the concentrations of Cu and Zn were higher in comparison to concentrations measured at site Kozloduy. This was a result from the impact of the tributaries, which join the Danube before the sites Vidin and Silistra. The profile was clearly expressed by the concentration values obtained both for parasites and water (see **Figure 4.1**; Litheraty *et al.* 2009). As mentioned above, the relationship between the Zn concentrations in the parasite and the source (water) were the same at both studied sites. This fact fulfils one of the criteria characterizing the ideal sentinel organism as summarized by Sures (2003). The lower Cu and Zn levels at Kozloduy were probably due to dilution along the river stretch after the confluence of the River Timok in the Danube. The decreasing concentration of As, Cd, Mn and Pb in parasites samples obtained in downstream direction can be also related to the dilution factor along the river stretch after the influence of the tributaries such as Rivers Sava, Tisa and Timok. Worth noticing is the reported during the JDS2 highest Ni concentration at the confluence of Timok River. The uptake of this element was found to be lower for *P. laevis*. However, its concentrations in barbel's muscles were significantly higher at Vidin, situated close to the inflow of Timok. The exposure duration of the pollutants can be evaluated as suggested by Sures *et al.* (1999*a*) with the help of ratios between the elements concentrations of host tissues (e.g. muscle) and

parasite. Accordingly, in this part of Danube a permanent contamination with nickel appears to occur, as fish muscle characterizes with slower uptake kinetics. It should be noted that a continuous Ni pollution over longer time is required for reaching such high saturation in the host's tissue.

During the Danube Surveys the main focus was on element concentrations in suspended particulate matter, to which heavy metals tend to adsorb and which is suggested to be more reliable for metal indications purposes than aqueous concentrations (Literathy *et al*. 2002; 2009). Following the profile of the distribution of heavy metals and As in SPM, published in the JDS2 report, the concentrations of As, Cd and Pb clearly decreased in downstream direction after km 1000 (see **Figure 4.4**). As previously mentioned, the As and Cd amounts in parasites also followed this profile. Unfortunately, the distribution patterns represented the conditions in 2007, while our data regards to monitoring performed in 2006. However, it could be suggested that over the period between both Danube surveys the conditions in this part of the River did not change drastically, since similar tendencies were observed. Additionally, for more detailed comparisons between contents in SPM and parasites, the raw data is required, because the published profiles did not deliver the needed resolution and it was not possible to assess fully the influence of tributaries like Timok and Russenski Lom.

The analysis on the longitudinal profile conducted in 2007 for the entire Danube River revealed some differences in element concentrations measured in *P. laevis* for lower Danube reaches compared to the upper one. In general, the distribution pattern of the accumulated by the parasite elements followed the longitudinal profile of SPM. It was clearly visualized by the profile obtained for As. The values measured for SPM in 2007 characterized with two humps along the river. The first was around km 2000 after the confluence of River Inn and the second at km 1000 behind the tributaries Tisa, Sava und Velika Morava. Similar results were obtained for As in parasite and host tissues, whereas increased concentrations were measured at the locality Vienna downstream (site JDS 13; 1930 km) followed by the Danube site Kozloduy in lower Danube (**Figure 4.2**). A decrease in As content, as reported for SPM, was observed along the river stretch between km 1930 and 1648 (between the site JDS 13 and the site JDS 32). As expected from the SPM profile, the elements Cd and Pb also showed highest values in lower Danube - the mean cadmium concentration at site Kozloduy was up to 16 times higher in comparison than those in the upper Danube sites. A parallel with the SPM profile could not be found for the essential elements, due to large fluctuations in the measured values. Therefore, for further detailed analyzes, the raw element data for SMP is required.

The long term monitoring (from summer 2004 to summer 2007) revealed a tendency of decreasing of Cd, Cu, Pb and Zn concentrations in parasites at site Kozloduy. The

improvement of water quality during this period was also obvious from the Cu concentrations at the confluence of Timok River. For example, in 2001 the reported aqueous Cu concentration was 163 µg/L, which was approximately five times higher compared with 2007 (34.5 µg/L) (Literathy *et al*. 2002; 2009). The descent of concentrations was also detectable in parasite samples from Kozloduy, situated more than 150 river kilometres downstream of the river Timok (see **Figure 4.3**). The enhancement of water quality regarding some of these heavy metals could be followed also in the chemical data published by the TNMN for the water column. According to the latter, at the monitoring point near to Kozloduy (Iskur), there was also a slight decrease in the Zn and Cu concentrations for the period 2003-2006 (**Table 4.2**). Unfortunately, such clear tendency was not found for the elements Cd and Pb. As mentioned above, the heavy metal monitoring in the water column does not always deliver a reliable picture of metal pollution in contrast to bioindicators. A reason for this is that the water phase is a highly dynamic system and the concentrations of diluted substances (metals) can vary between the sampling activities, although a pollution source exists. The monitoring performed by TNMN was based on a monthly assessment of element concentrations in the water and thus cannot be regarded as highly representative for the environment conditions. Using parasites, it was possible an obvious reduction of concentrations of elements Cd, Cu, Pb and Zn to be detected, which was suggested to be related to metallurgy industry in the catchments of the river Timok. The main reason for this was the constriction of mining in the region, which led to decreasing of pollution levels in the Timok River and its tributaries. The ore production at the open pit situated in Timok's catchment took place in the period from 1991 to 2002, after which the mining works were stopped. (Paunović *et al.* 2008).

Despite the observed deviations, the mean concentrations in *P. laevis* seemed to reflect the local conditions, where the fish were sampled. This deviation was considered to be related to the mobility of the fish, which might cause variation in the measured concentrations. However, such tendency is expected to occur even if other organisms (e.g. bivalves) are taken as sentinels. Even in the case of mussels are sampled from the same locality, the morphological differences of the riverine may lead to high deviation in the measured concentrations. The size and the age of the taken specimens play also a considerable role in the accumulation process, as already discussed in **Chapter 3**. Therefore, having this background and according to the obtained data, *P. laevis* can be regarded as a very appropriate tool in the field of metal monitoring. Its sentinel properties might be very useful for detecting and quantifying pollution sources from the industry and particulary for some highly toxic elements like As, Cd and Pb.

Summary, conclusions and future prospects

Summary

The main focus of the current thesis was to extend the knowledge about the use of fish parasites as bioindicators. On one hand the research emphasis was aimed at the faunistical and ecological aspects of parasite communities of barbel (*Barbus barbus*) in relation to environment conditions - the use of parasites as effect indicators (**Chapter 1**). On the other hand the thesis evaluated and reverified the acanthocephalan *Pomphorhynchus laevis* as accumulation indicator, while covering all critical aspects (as summarized by Sures, 2003) concerning its application - the effects of size and sex composition of its infracommunities (**Chapter 2**) as well as the seasonality (**Chapter 3**) on the metal uptake process in the parasite. Finally, the thesis delivered a detailed metal monitoring survey focused mainly on the lower Danube (**Chapter 4**), which was perforemed using the suggested barbel – *P. laevis* system.

The important results are summarized in the following:

Chapter 1: The endohelminth fauna of barbel correlates with water quality of the Danube River in Bulgaria.

- Infection of barbel with ten species of metazoan parasites including three trematodes, three acanthocephalans and four nematodes was observed in fish collected from three localities in the Bulgarian part of the river Danube between summer 2004 and summer 2007.
- New host records for three parasitic species – the nematode larvae of genus *Eustrongylides* sp. and *Hysterothylacium* sp. as well as the acanthocephalan *Leptorhynchoides plagicephalus* were recorded for first time for the host *Barbus barbus*.
- The most prevalent species was the acanthocephalan *Pomphorhynchus laevis*, which was also the dominant species of the intestinal component communities at all sampling sites.
- The second most frequent parasite at all Danube localities was *Rhabdochona hellichi*, which occurred in significantly higher numbers at the less polluted sites.
- The composition as well as the diversity characteristics of the parasite communities showed a clear correlation with the composition of the invertebrate fauna and water

quality – overall, the diversity of helminth communities increased with decreasing levels of nutrients and pollutants at all sampling sites.

Chapter 2: Is metal accumulation in *Pomphorhynchus laevis* dependent on parasite sex or infrapopulation size?

- From total twelve analyzed elements (As, Cd, Co, Cu, Fe, Mn, Mo, Ni, Pb, Sn, V, Zn) five (As, Cd, Cu, Pb and Zn) were detected in significantly higher concentrations in the acanthocephalan *P. laevis* compared to its host tissues (muscle, intestine, liver)
- According to the calculated mean bioconcentration factors, three more elements (Co, Mn, V) usually were with higher concentrations in *P. laevis*.
- Comparisons between high and low infected fish revealed significant differences only for V with higher concentrations for the heavily infected group.
- Concerning sex specific metal accumulation V and Zn showed significant differences (V, at $p < 0.05$; Zn, at $P = 0.05$), with higher levels in females of *P. laevis* each.

Chapter 3: Seasonal differences of metal accumulation in *Pomphorhynchus laevis* and its definitive host *Barbus barbus*.

- The concentrations of As, Cd, Cu, Pb and Zn were significantly higher in the parasite samples presented than in the host's tissues.
- These elements showed also a clear seasonal pattern, while the concentrations in the fish tissues remained similar in spring, summer and autumn.
- Seasonal variation in the mean individual weight of parasite infrapopulations, whereas the infrapopulations in autumn characterized with significantly lower mean individual weight than these in spring and summer - sign for predominant young specimens.
- Composition pattern of all accumulated elements reflected the pattern of the mean individual weight over the year - the highest concentrations obtained for As, Cd, Cu, Pb and Zn in *P. laevis* were found in autumn, followed by spring and summer.
- Concentration of Cd and Pb in *P. laevis* correlated negatively with the mean worm weight.
- Significant differences for the metals Cd and Pb were found, when the concentration in worms from summer and autumn were compared.

Chapter 4: Application of acanthocephalan *Pomphorhynchus laevis* from its host barbel (*Barbus barbus*) as metal indicator in the Danube River.

- The elements As, Cd, Cu, Pb and Zn were found to be significantly higher accumulated in the parasite compared to its host tissues.
- The concentrations of Cd, Cu, Pb and Zn in *P. laevis* exceeded the concentrations reported for the water column at the selected sampling sites.
- The longitudinal pattern of As, Cd, Cu, Pb and Zn in the parasite samples corresponded to the background available data (for water and suspended particulate matter) along the Danube River in Bulgaria.
- The comparisons between upper and lower Danube, performed in summer 2007, demonstrated increased concentrations of As, Cd and Pb in the lower Danube, whereas As showed also a peak in the upper reaches. Their concentration pattern reflected the pattern of suspended particular matter obtained during the 2nd Joint Danube Survey performed also in summer 2007.
- The long term monitoring at sampling site Kozloduy (685 km) showed progressive decrease in the concentrations of Cd, Cu, Pb and Zn for the period summer 2004 to summer 2007, while the As contents remained similar.

Conclusions and future prospects

The collective data suggests that the fish parasites can be successfully used to characterize the ecosystems health and integrity. Following the alternations in composition and diversity of their communities, it was possible to detect differences in the environmental conditions between investigated sampling sites. Therefore fish parasites can be efficiently applied as effect indicators in the aquatic monitoring. The last published data on parasite fauna of barbel for the Bulgarian part of Danube River was from 1960 and 70-ties (Kakacheva-Avramova, 1962, 1977; Margaritov, 1959, 1966). The results obtained from the present investigation showed that the fauna composition completely differed from the one reported 40-50 years ago. The disrepancy can be associated with the changed/disturbed ecological conditions in the investigated river stretch.

Further investigations aimed on other river stretches in middle and upper part of the river and on other aquatic habitats as well should be performed for the sake of the future implementation of fish parasites as effect indicators in the Danube River and in the hydrobiological praxis as well. The faunistical data from the different parts of the Danube should be compared and subsequently correlated with the local abiotic and biotic data, in

order to confirm profoundly the relationship between parasites and environmental conditions. Furthermore, different host-parasite systems should be also be included in such surveys and studied from a bioindication perspective.

A major goal of the current thesis was filling in on the lack of knowledge regarding the application of fish acanthocephalans as accumulation indicatiors. The obtained results suggested that the size and the sex composition of acanthocephalan's infrapopulations play no considerable role in the metal uptake process. Therefore, in metal monitoring surveys, especially in those aimed on toxic elements such as As, Cd and Pb, these aspects should not be taken into account. Worth noticing is that the results regarded the acanthocephalan *P. laevis*. If other acanthocephalan species are taken as accumulation indicators, these aspects should be studied in order to confirm the tendencies obtained in the thesis.

On the other hand the results revealed a seasonal pattern in the metal uptake, which was found to be dependent on the stage of acanthocephalan's development in the final host. Thus, the seasonality of transmission of *P. laevis* under the local climate conditions should be considered in order to make our monitoring surveys more precise. In some geographical regions, where the seasonality of transmission is not clearly pronounced, the seasonal factor can be suppressed. Of course, the sentinel features of fish acanthocephalans should also be investigated under different climate conditions, in order to select the proper sampling periods for metal monitoring surveys. The same should be done with other acanthocephalan species, if they are taken as metal indicators.

With the help of the background metal monitoring data delivered by the International Commission for Protection of Danube River (ICPDR), it was confirmed that the levels of the elements accumulated in *P. laevis* corresponded to these in the environment. The pollution profile in the Danube River basin, obtained during the both Joint Danube Surveys (in 2001 and 2007), was additionally confirmed by the concentrations measured in the parasites. Desipite the mobility of the fish host, the results of this thesis suggest the fish-parasite system is a perfect model in the field of ecological monitoring. However, future detailed analysis and correlations between the raw data from the JDS2 and obtained parasite data are required. Unfortunately, this data concerning the element concentrations in water, SPM, sediment and biota is still not available.

Regarding the practical use of a fish-parasite system as sentinel, the first step was made during the second Joint Danube Survey in 2007, where fish muscle tissue was analyzed. During the thesis I had the opportunity to contribute to the survey with metal analysis carried out on barbel – *P. laevis* system. The combinded results suggest that the additional use of fish acanthocephalans as sentinels represents a more powerful approach in heavy metal

monitoring surveys, due to the higher accumulation capacity of acanthocephalans compared to fish muscle. Consequently, in future monitoring programs the fish parasites should be accessorily implemented as sentinels, especially in large and complex lotic systems like Danube River.

Zusammenfassung

Indikationsvermögen von Fischparasiten zur Beurteilung des ökologischen Zustandes aquatischer Habitate

Hintergrund

Das im letzten Jahrzehnt steigende Interesse an Helminthen als potentielle Bioindikatoren für die Belastung und Verschmutzung von Gewässern mit Schwermetallen hat zu einer verstärkten Forschung auf dem Gebiet der ökologischen Parasitologie geführt. Die Möglichkeit, Fischparasiten als Indikatoren für die Beurteilung der Wasserqualität zu nutzen wurde in den letzen Jahren intensiv erforscht (MacKenzie *et al*. 1995; Kennedy, 1997; Lafferty, 1997; Overstreet, 1997; Sures *et al.* 1997*b*; Valtonen *et al.* 1997; Lafferty und Kuris, 1999; Sures *et al.* 1999*a*; Sures, 2001). Parasiten können als Effektindikatoren (Valtonen *et al*. 1997; Sures, 2001), und als Akkumulationsindikatoren (Sures *et al*. 1999*a*; Sures, 2001) benutzt werden. Dabei übertreffen sie sogar die Bioindikationseigenschaften der bislang bekannten freilebenden Organismen. Bei den freilebenden Bioindikatoren wurden vor allem die Akkumulationseigenschaften sowie Änderungen in der Physiologie und Ethologie erforscht, die durch Veränderung der Umweltqualität entstanden sind (Gunkel, 1994).

Eine Möglichkeit für einen Einsatz von Parasiten in der Effektindikation, liegt in der Erfassung der Diversität und der Veränderung von Parasitengemeinschaften. Bei dieser Form der Effektindikation liefern die Organismen durch ihre An- oder Abwesenheit Informationen über den physikalisch-chemischen Zustand der Umwelt (Sures, 2003). Eine Untersuchung der Diversität, Struktur und Dynamik der Parasitengemeinschaften hilft den Zustand und die Veränderlichkeit natürlicher Ökosysteme zu erfassen. Um die Auswirkungen, die Umweltkontaminationen auf Parasitengemeinschaften ausüben zu erfassen, müssen viele Aspekte berücksichtigt werden, wie z.B. die Dynamik und Eingliederung des Fischwirtes in das Nahrungsnetz (Marcogliese und Cone, 1997), die Beziehung und die Wechselwirkung zwischen den Parasiten (Overstreet, 1997) sowie die Ab- und Anwesenheit der Zwischenwirte. Außerdem beeinflussen Faktoren wie der pH-Wert (Marcogliese und Cone, 1997) und der Grad der Eutrophierung (Valtonen *et al.* 1997) direkt oder indirekt die Abundanz, die Verteilung und die Struktur von Parasitenpopulationen.

Wie bereits erwähnt, können Fischparasiten auch als Akkumulationsindikatoren verwendet werden. Durch ihre Fähigkeit, verschiedene Substanzen in ihrem Gewebe zu akkumulieren, liefern sie als Akkumulationsindikatoren Informationen über den chemischen Zustand ihrer

Umwelt. Gegenwärtig ist bekannt, dass nicht nur freilebende Organismen, wie z.B. Krebse und Muscheln, Schwermetalle in ihrem Gewebe akkumulieren können, sondern auch Parasiten. Und zwar in einem Maß, das die Konzentrationen in den Geweben des Wirtes oder der Umwelt, um ein Vielfaches übersteigt.

Durchgeführte Untersuchungen an parasitischen Nematoden deuten darauf hin, dass diese Helminthen nicht als Akkumulationsindikatoren geeignet sind, da die Anreicherung der Metalle zu niedrig ist (Sures *et al.* 1994*b*; Sures *et al.* 1998; Szefer *et al.* 1998; Baruš *et al.* 1999*a*,*b*). Cestoden dagegen scheinen vielversprechendere Akkumulationsindikatoren zu sein (Riggs *et al.* 1987; Turčeková und Hanzelová, 1996; Sures *et al.* 1997*c*; Tenora *et al.* 1997; Baruš *et al.* 2000; Sures *et al.* 2002). Anhand experimenteller Daten lässt sich ihre Akkumulationsfähigkeit höher einstufen als die freilebender Organismen. Die hinsichtlich ihrer Bioakkumulationsfähigkeiten am besten untersuchte Parasitengruppe sind die Acanthocephalen (vgl. z.B. Sures 2003, 2004*a,b*). Es gibt nicht nur eine Reihe von Freilandsstudien (Sures *et al.* 1994*a*,*b*,*c*; Sures und Taraschewski, 1995; Sures *et al.* 1997*a*, 1999*b*; Sures und Reimann, 2003), sondern auch Laboruntersuchungen (Siddall und Sures, 1998; Sures und Siddall, 1999; Zimmermann *et al.* 1999; Scheef *et al.* 2000; Sures *et al.* 2000*b*; Sures und Siddall, 2001, 2003; Sures *et al.* 2003) zur Schwermetallakkumulationskapazität von Acanthocephalen. Die Metallanreicherung bei adulten Acanthocephalen kann einige tausend Male höher sein als in den Geweben ihres Endwirtes. Der Akkumulationsprozess fängt dabei unmittelbar nach der Infektion des Endwirts an und erreicht in 4-5 Wochen seine Gleichgewichts-Konzentration. Untersuchungen an Larvenstadien weisen darauf hin, dass diese Stadien noch nicht in der Lage sind, Metalle in hohen Konzentrationen zu akkumulieren (Sures, 2003). Auch im Vergleich zu etablierten, freilebenden Bioindikatoren ist die Biokonzentration von Cd und Pb in Acanthocephalen um ein Vielfaches höher, wie der unmittelbare Vergleich der Metallanreicherung in *Acanthocephalus lucii* und der Dreikantmuschel, *Dreissena polymorpha*, zeigt (Sures *et al.* 1997*a*, 1999*b*). Trotz der enormen Akkumulationskapazität von Fischacanthocephalen bestehen diverse unerforschte Aspekte, die potenziell ihre praktische Anwendung im Bereich des Metallmonitorings kritisch machen könnten. Wie von Sures (2003) zusammengefasst, sollten noch die Auswirkung des Alters und der Größe von Parasiten sowie Effekte der Saisonalität und der Reproduktionsaktivität auf die Metallaufnahme gründlich erforscht werden, um die Acanthocephalen als Akkumulationsindikatoren einsetzen zu können.

In der vorliegenden Arbeit wurde versucht, die oben genannten Aspekte bezüglich der Anwendung von Fischparasiten als Indikatoren zu erfassen. Daraus leiten sich die folgenden Arbeitshypothesen und Schwerpunkte ab:

(1) Die Zusammensetzung der Fischparasiten-Gemeinschaft korreliert mit dem Verschmutzungsgrad in den Flussabschnitten, aus welchen die Fische stammen.
Die Fischparasiten reagieren auf die veränderten Umweltbedingungen mit einer Änderung in ihrer Diversität und Artenzusammensetzung. Die Verschmutzung (chemische oder physikalische) kann direkt oder indirekt die Fischparasitenpopulationen beeinflussen. Der direkte Einfluss zeichnet sich durch eine letale Reaktion der Larven- oder Adultstadien aus. Bei indirektem Einfluss handelt es sich um letale Effekte auf die Zwischen- oder Endwirte, wobei die Effizienz der Parasitentransmission verhindert wird. Zudem könnte die Verschmutzung die Wirtsphysiologie insofern beeinflussen, dass Wirt und Parasit zusätzlich unter diesem externen Stress leiden. In beiden Fällen führt die Kontamination zu Änderungen in der Artenzusammensetzung und der Diversität von Parasitenpopulationen.

(2) Die Infrapopulationsgröße und geschlechtsspezifische Metallanreicherung in dem Acanthocephalen Pomphorhynchus laevis *und dessen Endwirt* Barbus barbus.
Die Fischacanthocephalen sind fähig die Metallgehalte in den Wirtsorganen zu reduzieren, wobei die Konzentrationen im Wirtsgewebe negativ mit der Anzahl der Würmer im Darm korreliert (Sures und Siddal, 1999; Sures *et. al.* 2003). Für den Fall, dass Fischacanthocephalen als Metallindikatoren verwendet werden, sollte untersucht werden, ob die Infrapopulationsgröße berücksichtigt werden muss. Parallel könnte, bedingt durch den unterschiedlichen Metabolismus, ihre Geschlechterzusammensetzung auch eine Rolle bei der Metallaufnahme spielen. Zusätzlich könnten die rein morphologischen Unterschiede zwischen den Geschlechtern auch einen Einfluss auf den Akkumulationsprozess aufweisen. Dies könnte durch unterschiedliche Oberfläche- Volumen-Verhältnisse verursacht werden.

(3) Jahreszeitliche Unterschiede bei der Metallaufnahme in Pomphorhynchus laevis *und dessen Endwirt* Barbus barbus.
Eine Saisonalität bei der Metallaufnahme in den derzeit verwendeten freilebenden Indikatororganismen (wie z.B. Muscheln) ist bereits bekannt. In den meisten Fällen sind die Unterschiede durch deren Reproduktionszyklen geprägt. Ein Grund dafür ist hauptsächlich die Änderung in der Metabolismusaktivität vor und nach der Gametenfreisetzung (Luoma und Rainbow, 2008). Ähnliche Tendenzen sind auch bei den Fischacanthocephalen während ihrer

Entwicklung im Darm des Endwirtes zu erwarten. Dadurch besteht ein Bedarf, bestimmte Beprobungszeiträume zu berücksichtigen, damit der Faktor Saisonalität vermindert werden kann.

(4) Die Metallkonzentrationen in dem Acanthocephalen Pomphorhynchus laevis *spiegeln die Konzentrationen in der Umwelt wider.*
Um einen Akkumulationsindikator effektiv für Metallmonitoringszwecke verwenden zu können, müssten dessen Gewebekonzentrationen jene der Umwelt widerspiegeln (zusammengefasst von Sures, 2001). Darüber hinaus sollten die Fischacanthocephalen eine realistische Information über den Metallbelastungsgrad in den entsprechenden aquatischen Habitaten liefern.

Um die Schwerpunkte der Dissertation abdecken zu können, wurden die folgenden Methoden und Materialen angewendet:

Durchführung der Arbeit

In dieser Forschungsarbeit wurde eine Freilandstudie zur Zusammensetzung der Fischparasitengemeinschaft an verschiedenen Standorten im Verlauf der Donau durchgeführt. Zwischen 10 und 30 Barben (*Barbus barbus*) der jeweiligen Probestellen wurden im Zeitraum Sommer 2004 bis Sommer 2007 auf ihre Parasitozönosen hin untersucht. Die Fische wurden von Berufsfischern entlang der Donau bezogen, wobei ein Teil (im Sommer 2007) während der zweiten wissenschaftlichen Donau Expedition (JDS2) gefischt wurde. Die Fische wurden im eingefrorenen Zustand in das Labor gebracht, wo sie einer vollständigen parasitologischen Untersuchung unterzogen wurden (vgl. z.B. Sures *et al.* 1999*c*). Aus den Daten zur Befallssituation der Fische, unter spezieller Berücksichtigung von Helminthen, wurden dann die mittleren Diversitäts- und Dominanzindices (vgl. z.B. Magurran, 1988; Sures *et al.* 1999*c*) berechnet, so dass objektive Größen resultierten, die einen Vergleich der Fischparasitozönosen zwischen den Probestellen sowie zwischen den Jahreszeiten erlauben. Zusätzlich wurden die Resultate der Fischparasitozönose in Korrelation zu den entsprechenden Makrozoobenthos-Daten und den physikalisch-chemischen Wasserparametern gesetzt. Diese Hintergrunddaten wurden von der Datenbank der Internationalen Kommission zum Schutze der Donau (ICPDR) entnommen.
Zusätzlich wurden die Parasiten (insbesondere Acanthocephalen) wie auch verschiedene Fischgewebe (Muskel, Darm und Leber) im Labor auf ihren Schwermetallgehalt hin

untersucht. Dazu wurden die Proben mittels Mikrowellenaufschluss in Lösung gebracht (Zimmermann *et al.* 2001). Anschließend wurden die Metallgehalte mit Hilfe der Massenspektrometrie (ICP-MS) gemessen. Die Konzentrationen der Elemente As, Cd, Co, Cu, Fe, Mn, Mo, Ni, Pb, Sn, V und Zn wurden analysiert. Um die Akkumulationskapazität von Fischacanthocephalen zu ermitteln, wurden mittlere Biokonzentrationsfaktoren für die gemessenen Elemente wie folgt berechnet (nach Sures *et al.* 1999*a*): $C_{[P.laevis]}$ / $C_{[Wirtsgewebe]}$; $C_{[P.laevis]}$ / $C_{[Wasser]}$.

Wichtige Ergebnisse und Erkenntnisse

Die oben genannten Schwerpunkte und Arbeitshypothesen der Dissertation wurden einzeln jeweils in Unterkapiteln dargestellt und diskutiert.

1) Die Zusammensetzung der Fischparasiten-Gemeinschaft korreliert mit dem Verschmutzungsgrad der Flussabschnitte, aus welchen die Fische stammen.

Insgesamt wurden 407 Barben aus dem Zeitraum Sommer 2004 bis Sommer 2007 parasitologisch untersucht. Die Fische stammten aus drei Beprobungsstellen in dem bulgarischen Abschnitt der Donau und wurden jeweils im April, Juli und Oktober entnommen. An den Probestellen Vidin (834 km) und Kozloduy (685 km) wurden jeweils 165 bzw. 193 Barben in dem gesamten Zeitraum untersucht und an der Stelle Silistra (375 km) insgesamt 49 Barben im Jahr 2006 und 2007.

Die erhobene Parasitenfauna umfasste 10 Endohelminthenarten - 3 Trematodenarten (Metacercarie von *Diplostomum spathaceum* in den Augenlinsen, *Posthodiplostomum cuticola* auf der Haut, *Metagonimus yokogawai* auf den Schuppen), 3 Acanthocephalenarten (*Pomphorhynchus laevis*, *Acanthocephalus anguillae* und *Leptorhynchoides plagicephalus* im Darm) und 4 Nematodenarten (Adulten von *Rhabdochona hellichi*, *Pseudocapillaria tomentosa* und Larven von *Hysterothylacium* sp. im Darm und Larven von *Eustrogylides* sp. in der Leibeshöhle). Der Acanthocephale *L. plagicephalus* und die Larven von den Nematoden *Eustrongylides* sp. und *Hysterothylacium* sp. wurden zum ersten Mal für den Wirt Barbe beschrieben.

Die dominante Parasitenart an allen drei Probestellen war der Acanthocephale *P. laevis*, der mit eine Befallsrate von fast 100 % vorkam. Der zweithäufigste Parasit war der Nematode *R. hellichi* dessen Prävalenz und Befallsintensität an den Stellen im Unterlauf (Kozloduy und Silistra) mit einer besseren Wasserqualität anstieg. Im Gegensatz dazu kam der Nematode *Eustrgylides* sp. zusammen mit *R. hellichi* mit gleichen Befallsraten an der Probestelle im

Oberlauf (Vidin) vor, wobei im Lauf der Donau seine Befallsrate und Intensität absanken. Die Verteilungsmuster dieser Parasitenarten können auf den Verschmutzungsgrad (Eutrophierungsgrad) bezogen werden, wenn davon ausgegangen wird, dass diese Arten unterschiedliche Zwischenwirte benötigen. Als Zwischenwirt für *R. hellichi* dienen Köcherfliegenlarven (Trichoptera) von der Gattung *Hydropsyche* (Moravec, 1995), deswegen war die niedrigste Prävalenz dieser Art an der Stelle Vidin zu finden- auf Grund der niedrigen Abundanz des Zwischenwirtes. Der Fakt spiegelt den höheren Eutrophierungs- und Verschmutzungsgrad wider, welcher von der Internationalen Kommission zum Schutz der Donau für diesen Flussabschnitt ermittelt wurde. Die Larven von *Hydropsyche* sp. werden im Saprobiensystem mit einem Indikationswert zwischen 2.1 und 2.3 eingestuft (Moog, 1995).
Im Gegensatz zu *R. hellichi* erfolgt die Entwicklung der Nematoden der Gattung *Eustrogylides* sp. über Oligochaeten (*Lumriculus variegatus*, *Tubifex tubifex*, *Limnodrilus* sp.) als Zwischenwirt (Morevec, 1994), die mit einem Indikationswert von über 3 organisch- und chemisch-belastete aquatische Habitate charakterisieren. Es wurde gefolgert, dass das Vorkommen von diesen Parasitenarten abhängig von der Belastung war.
Die berechneten Diversitätsindizes (Brillouin- und Schannon-Wiener- Index) unterstützten zusätzlich die Korrelation mit dem Belastungsgradienten. Die niedrigsten Werte wurden an der Probestelle Vidin gemessen, während die unteren zwei Stellen deutlich höhere Werte aufwiesen. Das Belastungsprofil im bulgarischen Abschnitt der Donau konnte zudem durch die chemischen Hintergrunddaten der ICPDR belegt werden (Literathy *et al*. 2002, 2009; TNMN, 2009).
Somit konnte gezeigt werden, dass die Zusammensetzung der Parasitenfauna mit den lokalen Gewässerbedingungen korreliert. Die Diversitätsindizes spiegelten auch den Belastungsgradient entlang der untersuchten Flussstrecke, wobei die Diversität hier als ein Maß für die allgemeine Ökosystemgüte betrachtet wird.

2) Die Infrapopulationsgröße und geschlechtsspezifische Metallanreicherung in dem Acanthocephalen Pomphorhynchus laevis *und dessen Endwirt* Barbus barbus.

Die Konzentrationen von 12 Elementen (As, Cd, Co, Cu, Fe, Mn, Mo, Ni, Pb, Sn, V, Zn) wurden mittels Massenspektrometrie mit induktiv gekoppeltem Plasma (ICP-MS) im Darmparasiten *Pomphorhynchus laevis* und in den Geweben (Muskel, Darm, Leber) seines Wirtes *Barbus barbus* analysiert. Der Zweck der Untersuchung war, eventuelle Anreicherungsunterschiede, sowohl zwischen gering und stark infizierten Fischen, als auch zwischen beiden Geschlechtern von *P. laevis* festzustellen. Dafür wurden 30 mittelgroße Fische im Oktober 2006 von einer Probestelle bei Flusskilometer 685 an der bulgarischen

Flussbank der Donau entnommen und entsprechend ihrer Befallsintensität mit Acanthocephalen eingeteilt. Fische (n=9) mit einer Befallsintensität weniger als 20 Würmer wurden der Gruppe Gering Infiziert zugeteilt. Eine zweite Gruppe (n=9) mit einer Befallsintensität von mehr als 100 Parasiten wurde der Gruppe Stark Infiziert zugeordnet. Anhand dieser beiden Gruppen wurden potenzielle Akkumulationsunterschiede in den Wirtsgeweben und den Acanthocephalen zwischen gering und stark infizierten Fischen untersucht. Eine weitere dritte Gruppe (n=8) von Barben mit einer Befallsintensität zwischen 66 und 89 Würmern wurde ausselektiert. Die Gruppe wurde für die Erfassung geschlechtsspezifischer Unterschiede in der Metallanreicherung verwendet.

Die Elementzusammensetzung im Wirt-Parasit-System wies einen signifikant stärkeren (bis zu 1070 höheren) Anreicherungsgrad von As, Cd, Cu, Pb und Zn im Parasiten im Vergleich zu den Wirtsgeweben auf. Gemäß den berechneten Biokonzentrationsfaktoren wurden drei weitere Elemente (Co, Mn, V) mit einer höheren Konzentration in *P. laevis* gefunden. Die Vergleiche zwischen stark und leicht infizierten Fischen zeigten weder in den Wirtsgeweben noch im Parasiten signifikante Unterschiede. Die einzigen Anreicherungsunterschiede wurden für das Element Vanadium in Parasitenproben und Fischleber gefunden, wobei die stark infizierte Gruppe höhere Gehalte aufwies. Zusammenfassend blieb die Konzentration von den in *P. laevis* stark akkumulierten Elementen (As, Cd, Cu, Pb und Zn) unabhängig von der Befallsintensität.

Zwischen den beiden Geschlechtern von *P. laevis* wurden signifikante Unterschiede nur für die Elemente V ($p < 0.05$) und Zn ($p \approx 0.05$) festgestellt. Wobei die Weibchen jeweils höhere Gehalte aufwiesen.

Die Ergebnisse deuten darauf hin, dass *P. laevis* gut geeignet für Metallmonitoringstudien ist, da die Infrapopulationsgröße und Geschlechterzusammensetzung keinen großen Einfluss auf den Akkumulationsprozess im Wirt-Parasit-System ausüben. Diese Aspekte zusammen mit der enormen Akkumulationskapazität, besonders für toxische Metalle wie Cd and Pb, stützen den Vorschlag für die Verwendung von *P. laevis* als Akkumulationsindikator.

Die Arbeitshypothese, dass die Metallanreicherung im Wirt-Parasit System von der Größe und Geschlechterzusammensetzung der Parasiteninfrapopulationen abhängig ist, konnte nicht vollständig bestätigt werden. Bis auf die Elemente V und Zn, die noch vom Wirtsmetabolismus als essentielle Metalle (wie z.B. Zn) beeinflusst werden, war die Elementzusammensetzung in Parasiten ähnlich.

3) Jahreszeitliche Unterschiede bei der Metallaufnahme in Pomphorhynchus laevis *und dessen Endwirt* Barbus barbus.

Um die Wirkung der Saisonalität auf die Metallaufnahme in den Acanthocephalen zu erforschen, wurden die Gehalte der Elemente As, Cd, Co, Cu, Fe, Mn, Mo, Ni, Pb, V und Zn in dem gewählten Wirt-Parasit System analysiert. Die Untersuchung deckte Frühjahr, Sommer und Herbst bzw. die Monate April, Juli und Oktober 2006 ab, wobei acht mittelgroße Barben jeweils pro Saison von einer Probestelle (Kozloduy, 685 km) entnommen wurden. Nach der parasitologischen Untersuchung wurde die Anzahl der Acanthocephalen und das Frischgewicht deren Infrapopulationen erhoben. Zusätzlich wurde die mittlere individuelle Masse von *P. laevis* für jede Barbe berechnet, als das Infrapopulationsgewicht geteilt durch die Anzahl der Würmer. Der Zweck dieser morphologischen Berechnung war Informationen über den Entwicklungszustand der Acanthocephalen im Endwirt zu erhalten. Darüber hinaus bringt die individuelle Wurmmasse auch weitere Erkenntnisse bezüglich der Oberflächen – Volumen-Verhältnisse von Parasiten, die eine entscheidende Rolle bei der Metallaufnahme spielen können ähnlich wie bei freilebenden Metallindikatoren wie z. B. Muscheln (Luoma und Rainbow, 2008).

Wie erwartet, waren die Konzentrationen der Elemente As, Cd, Cu, Pb und Zn im Parasiten im Vergleich zum Wirtsgewebe (Muskel, Darm und Leber) signifikant höher. Diese Elemente zeigten auch einen ähnlich ausgeprägten jahreszeitlichen Konzentrationsverlauf. Die höchsten mittleren Konzentrationen wurden im Herbst und die niedrigsten im Sommer nachgewiesen. Die Frühjahrswerte lagen im Bereich zwischen den Werten von Herbst und Sommer. Die Tendenz wurde zusätzlich statistisch für die toxischen Metalle Cd und Blei geprüft- deren Gehalte waren im Herbst signifikant höher. Die Konzentration dieser Metalle korrelierte negativ mit dem mittleren individuellen Wurmgewicht, und spiegelte zudem das jahreszeitliche Konzentrationsprofil der akkumulierten Elemente wider. Die berechnete mittlere Parasitenmasse war im Vergleich zu Frühjahr und Sommer im Herbst signifikant niedriger. Ein Zeichen dafür, dass die Achanthocephalen-Infrapopulationen im Herbst überwiegend aus jüngeren Individuen besteht. Dies beeinflusste die Wurmgehalte, da sich jüngere Individuen in der Wachstumsphase durch einen entsprechend intensiveren Metabolismus auszeichnen. Deswegen waren die mittleren Konzentrationen von As, Cd, Cu, Pb und Zn im Herbst am höchsten. Das höhere Oberflächen – Volumen-Verhältnis der jüngeren Acanthocephalen beeinflusste zusätzlich die Metallaufnahme, so wie für freilebende Metallindikatoren bereits beschrieben. Dieser Aspekt muss auch bei dem Metallaufnahmemechanismus der Acanthocephalen berücksichtig werden, da die Akkumulation durch die Tegumentoberfläche erfolgt. Zusätzlich brachte die Untersuchung

Erkenntnisse über die Saisonalität der Entwicklung von *P. laevis* unter den spezifischen klimatischen Bedingungen im Unterlauf der Donau. Das ausgeprägte Kontinentalklima beeinflusst den Lebenszyklus und die Übertragung der Parasiten, wobei ein jährliches Muster deutlich zu sehen ist. Von den erhobenen Ergebnissen zur Morphologie und Elementzusammensetzung der Acanthocephalen wurde eine grobe Schätzung der Lebensdauer von *P. laevis* im Darm des Endwirtes *B. barbus* erzeugt. Die Lebensdauer beträgt höchst wahrscheinlich 7-8 Monate.

Zusammenfassend wird deutlich, dass die Metallanreicherung in *P. laevis* von der Saisonalität bzw. von den Entwicklungsstadien der Parasiten abhängig ist.

4) Die Metallkonzentrationen im Acanthocephalen Pomphorhynchus laevis *spiegeln die Konzentrationen in der Umwelt wider.*

Die Metallmonitoringstudie mit Hilfe von Fischacanthocephalen wurde überwiegend entlang des bulgarischen Donauabschnitts durchgeführt. Zuerst wurde versucht ein Längsprofil der gewählten Flussstrecke zu erzeugen. Aus diesem Grund wurden im Sommer 2006 jeweils acht Barben von drei Probestellen entlang der Donau (Vidin, 834; Kozloduy, 685; Silistra, 375 km) untersucht. Zusätzlich wurde eine Langzeitmonitoringstudie im Unterlauf der Donau durchgeführt, die vier Jahre umfasste. Die Studie wurde an der Probestelle Kozloduy durchgeführt, die als Referenzstelle im Unterlauf gewählt wurde. Von dieser Stelle wurden in jedem Sommer in dem Zeitraum 2004 bis 2007 acht Fische entnommen. Im Jahr 2007 wurde noch eine weitere Studie durchgeführt, welche für einen Vergleich zwischen dem Ober- und Unterlauf der Donau diente. Für diesen Zweck wurden von vier Probestellen in Mitteleuropa Barben beprobt und mit der Probestelle Kozloduy im Unterlauf verglichen. Die Fische von Mitteleuropa stammten aus Probestellen in der Nähe von Wien (1930 km), Bratislava (1869 km), Szob (1707 km) und Budapest (1648 km) und wurden während der zweiten Donau Forschungsexpedition (JDS2) im Sommer 2007 gefangen.

Die Acanthocephalen und die Fischgewebe (Muskel, Darm, Leber) wurden auf den Gehalt mehrerer Elemente (As, Cd, Co, Cu, Fe, Mn, Mo, Ni, Pb, V, Zn) analysiert und es wurden Gehaltprofile erstellt. Die Konzentrationen im Parasit – Wirt-System wurden sowohl mit den Wasserdaten im Zeitraum 2004-2007 (monatliches Metallmonitoring des Wasserkörpers im Rahmen der TransNational Monitoring Network Programm) als auch mit den Metalldaten (zu Wasser und Schwebstoffen (SPM)) von beiden Donauexpeditionen (JDS1 in 2001 und JDS2 in 2007) verglichen (Literathy *et al*. 2002, 2009; TNMN, 2009).

Von den analysierten Elementen As, Cd, Cu, Pb und Zn wurden im Vergleich zu den Wirtsgeweben wieder signifikant höhere Konzentrationen in *P. laevis* gefunden. Um einen

Vergleich zwischen den Konzentrationen im Parasit und im Wasser zu erlauben, wurden die mittleren Biokonzentrationsfaktoren bezüglich des Wassers berechnet. Damit zeigte sich ein höherer Anreicherungsgrad für die Schwermetalle Cd, Cu, Pb und Zn in den Acanthocephalen. Das Längsprofil dieser Elemente in den Parasiten spiegelte das Längsprofil der Metallgehalte des Wassers und der Schwebstoffe wider. Ähnliche Ergebnisse wurden auch für das Element As beobachtet. Generell senken sich die Gehalte von As, Cd, Pb im Lauf der Donau in Bulgarien ab. Die essenziellen Metalle Cu und Zn wiesen höhere Konzentrationen an der oberen (Vidin) und an der unteren Probestelle (Sillistra) auf. Der Grund dafür sind zwei Nebenflüsse (Fluss Timok und Fluss Russenski Lom), die als die größten Verschmutzungsquellen für Cu und Zn von der ICPDR bezeichnet werden.

Das durchgeführte Langzeitmetallmonitoring an der Stelle Kozloduy zeigte eine Verbesserung der Wasserqualität bezüglich der vom Parasit akkumulierten Metalle. Mit der Ausnahme von Arsen senkten sich die Gehalte von Cd, Cu, Pb und Zn im Zeitraum vom Sommer 2004 bis Sommer 2007 ab. Die Tendenz wird zum Teil von den Hintergrunddaten der ICPDR belegt.

Die Vergleiche zwischen Ober- und Unterlauf der Donau zeigen generell eine deutliche Metallgehalterhöhung im Unterlauf an der Probestelle Kozloduy. Besonders deutlich zeigt sich dies für die Elemente Cd und Pb. Die höheren Konzentrationen in der bulgarischen Donaustrecke stehen unter dem Einfluss zweier großer Nebengewässer (Tisa und Sava), die im Gesamtverlauf der Donau von km 1000 bis zum Donaudelta zu Belastungen durch Schwermetalle führen (Literathy *et al*. 2009). Für detaillierte Analysen waren noch zusätzliche Rohdaten der zweiten Donauforschungsexpedition erforderlich.

Die Metallgehalte im Parasit spiegeln die Konzentrationen in der Umwelt wider. Die beobachtete Tendenz in den Parasitengehalten wird von den Ergebnissen der unterschiedlichen Donaumonitoringprogramme bestätigt.

Schlussfolgerungen

Die durchgeführte faunistische Untersuchung im Rahmen der Dissertation lieferte eine neue Wirtsmeldung für drei parasitische Arten. Die Nematoden der Gattung *Eustrongylides* sp. und *Hysterothylacium* sp und der Acanthocephale *L. plagicephalus* wurden zum ersten Mal für den Wirt *B. barubs* beschrieben, wobei *Eustrongylides* sp. einer der häufigsten Vertreter im Unterlauf der Donau war. Die Parasitenfauna der Barbe wies beim Vergleich der derzeitigen Ergebnisse mit den zuletzt publizierten Daten aus den 1960-er und 70-er Jahren (Kakacheva-Avramova, 1962, 1977; Margaritov, 1959, 1966) generell große Unterschiede auf. Ein möglicher Grund dafür liegt bei den veränderten Bedingungen im Unterlauf der Donau in den

letzten 40 Jahren.

Die abgedeckten Aspekte bezüglich der Anwendung von Fischparasiten als Indikatoren unterstützen deren Einsatz im Bereich des aquatischen Monitorings sowohl als Effektindikatoren als auch als Akkumulationsindikatoren. Ihre Anwendung als Effektindikatoren war von der Artenzusammensetzung unterstützt, da das Vorkommen der Parasiten zum Teil die Umweltbedingungen widerspiegelten. Zusätzlich lieferten die Diversitätindizes ähnliche Tendenzen, die den Belastungsgradienten in der Donau folgten.

In der Dissertation wurden diverse, bisher nicht untersuchte, Aspekte betrachtet, welche den Einsatz von Fischacanthocephalen als Akkumulationsindikatoren verhindern könnten (wie z.B. der Einfluss der Infrapopulationsgröße, der Geschlechterzusammensetzung und der Saisonalität auf die Metallanreicherung). Aus den erhobenen Ergebnissen können folgende Rückschlüsse gezogen werden:

- Die Infrapopulationsgröße übt keinen großen Einfluss auf den Metallgehalt in *P. laevis* aus. Darüber hinaus sollte die Befallsintensität bei Metallmonitoringsstudien mit der Hilfe von Fischacanthocephalen nicht berücksichtig werden.
- Die Geschlechterzusammensetzung der Parasiteninfrapopulationen muss nicht in Betracht gezogen werden, da beide Geschlechter ähnliche Tendenzen bei der Metallanreicherung gezeigt haben.
- Der Metallanreicherungsprozess ist von der Saisonalität bzw. vom Parasitenentwicklungsstadium im Endwirt abhängig. Darüber hinaus sollten bei den Beprobungszeiten unter anderem die lokalen klimatischen Bedingungen berücksichtigt werden.

Die Fischacanthocephalen, konkret *P. laevis*, scheinen sehr vielversprechend für Metallindikationszwecke zu sein. Die enorme Akkumulationskapazität besonders für toxische Elemente wie As, Cd und Pb zeichnet sie als ein perfektes Werkzeug im Bereich des aquatischen Monitorings aus. Trotz der höheren Mobilität des Fischwirtes, spiegelten die Konzentrationen in *P. laevis* jene der Umwelt wider und lieferten ein anschauliches Bild über die Metallbelastung der Donau. Der erste Schritt für den Einsatz von Fischen als Metallindikatoren wurde während der zweiten Donauforschungsexpedition (JDS2) gemacht (Literathy *et al*. 2009). Im Rahmen der Dissertation wurde bewiesen, dass im Gegensatz zur alleinigen Analyse von Fischgewebe, die zusätzliche Anwendung von Fischparasiten als Metallindikatoren ein vielversprechenderes Verfahren darstellt.

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Appendix

Appendices are available on the enclosed CD-ROM

Appendix I	Parasitological data of barbel.
Appendix I-A	Prasitological data of barbel collected from sampling site Vidin.
Appendix I-B	Prasitological data of barbel collected from sampling site Kozloduy.
Appendix I-C	Prasitological data of barbel collected from sampling site Silistra.
Appendix II	Element concentrations in barbel tissues and *P. laevis* used in **Chapter 2**.
Appendix II-A	Element concentrations in host tissues and *P. laevis* of heavily infected barbels.
Appendix II-B	Element concentrations in host tissues and *P. laevis* of slightly infected barbels.
Appendix II-C	Element concentrations in host tissues and females and males of *P. laevis.*
Appendix III	Water temperature data at two localities of the Danube River for the period 2005-2006.
Appendix IV	Morphological data of *P. laevis* used in **Chapter 3**.

Appendix I. Parasitological data of barbel.

In the Appendix were used the following abbreviations:

N: Barbel's number in the particular sample
S: Season (1 – spring; 2 – summer; 3 – autumn)
W: Fish weight (g)
TL: Total length (cm)
ST: Standard length (cm)
BH: Body height (cm)
K: Condition factor

P.l.:	*Pomphorhynchus laevis*	n = number of parasites
L.p.:	*Leptorhynchoides plagicephalus*	n = number of parasites
A.a.:	*Acanthocephalus anguillae*	n = number of parasites
R.h.:	*Rhabdochona hellichi*	n = number of parasites
P.t.:	*Pseudocapillaria tomentosa*	n = number of parasites
E.sp.:	*Eustrongylides* sp.	n = number of parasites
H.sp.:	*Hysterothylacium* sp.	n = number of parasites
P.c.:	*Postodiplostomum cuticola*	+ = yes, 0 = no
D.s.:	*Diplostomum spathaceum*	+ = yes, 0 = no
M.y.:	*Metagonimus yokogawai*	+ = yes, 0 = no

Appendix I-A. Parasitological data of barbel collected from sampling site Vidin.

N	S	W	TL	SL	BH	K	*P.l.*	*L.p.*	*A.a.*	*R.h.*	*P.t.*	*E.sp.*	*H.sp.*	*P.c.*	*D.s.*	*M.y.*
1	2	417	37.5	30.6	6.5	0.79	125	0	0	0	0	0	0	0	0	0
2	2	1350	55.6	46.6	10	0.79	138	0	0	1	0	0	0	0	+	0
1	3	1335	54.5	44.3	10.7	0.82	168	0	0	0	0	0	0	0	+	0
2	3	1515	55	45.1	11.1	0.91	283	0	0	0	0	16	0	0	0	0
3	3	1503	53.9	45.8	11.9	0.96	231	0	0	0	0	0	0	0	+	0
4	3	740	43.3	35.4	8.8	0.91	4	0	0	1	0	0	0	0	0	0
5	3	520	41.3	33.7	8.6	0.74	191	0	0	3	0	0	0	+	0	+
6	3	1816	60.1	49.4	11.5	0.84	93	0	0	7	0	0	0	0	0	0
7	3	1752	56.6	47.3	12.7	0.97	54	0	0	0	0	0	0	0	0	0
8	3	443	39	31.7	7.9	0.75	76	0	0	0	0	0	0	0	0	0
9	3	435	37.6	30.8	7.3	0.82	154	0	0	0	0	1	0	0	0	0
10	3	332	33	27.3	604	0.92	128	0	0	0	0	0	0	+	0	+
11	3	336	33.1	26.8	6	0.93	26	0	0	0	0	0	0	0	0	0
12	3	1164	53.8	44.3	9.6	0.75	232	0	0	0	0	22	0	0	0	0
13	3	432	37.3	29.9	6.9	0.83	47	0	0	0	0	0	0	0	0	0
14	3	406	35.3	29.8	7.4	0.92	183	0	0	0	0	0	0	0	0	0
15	3	629	41.5	33.8	8.1	0.88	23	0	0	1	0	0	0	0	0	0
16	3	876	45.9	37.6	9	0.91	24	0	0	0	1	0	0	+	0	0
1	2	1685	54	46.6	11.7	1.07	223	0	0	0	0	7	0	0	0	0
2	2	1095	49.7	42	9.7	0.89	329	0	0	0	0	5	0	0	0	0
3	2	400	37.4	30.4	6.9	0.76	312	0	0	0	0	1	0	0	0	0
4	2	575	40.4	33.7	7.6	0.87	166	0	0	0	0	0	0	0	0	0
5	2	314	34.9	28.5	6.2	0.74	229	0	0	0	0	0	0	0	0	0
6	2	255	30.9	26.2	5.9	0.86	64	0	0	0	0	1	0	+	0	0
7	2	300	33.4	28.4	6.5	0.81	133	0	0	0	0	0	0	0	0	0
8	2	800	45.5	39.5	8.5	0.85	344	0	0	0	0	0	0	0	0	+
9	2	602	42.6	35.5	8.3	0.78	37	0	0	0	0	1	0	+	0	0
10	2	1315	51.3	42.6	10.6	0.97	91	0	0	0	0	4	0	0	0	0

N	S	W	TL	SL	BH	K	*P.l.*	*L.p.*	*A.a.*	*R.h.*	*P.t.*	*E.sp.*	*H.sp.*	*P.c.*	*D.s.*	*M.y.*
11	2	335	35.6	29.2	5.6	0.74	243	0	0	0	0	0	0	0	0	0
12	2	1640	56.8	47.7	11.7	0.89	216	0	0	0	0	2	0	0	0	0
13	2	2145	63.7	52.2	11.7	0.83	191	0	0	3	1	0	0	0	0	0
14	2	645	42.9	35.3	8.4	0.82	219	0	0	0	0	0	0	0	+	0
15	2	238	31.2	25.8	5.6	0.78	54	0	0	0	0	0	0	0	0	0
16	2	1563	54.8	45.6	11.2	0.95	90	0	0	0	0	0	0	0	0	0
17	2	1521	55.4	45.6	10.3	0.89	401	0	0	0	0	24	0	0	0	0
18	2	1446	52.9	43.4	10.6	0.98	91	0	0	0	0	0	0	0	0	0
1	3	167	27.8	23	5.3	0.78	28	0	0	0	0	0	0	0	0	0
2	3	81	23.4	19	4.1	0.63	128	0	0	0	0	0	0	0	0	0
3	3	208	31.7	25.6	5.9	0.65	27	0	0	0	0	0	0	0	0	0
4	3	303	34.6	27.8	6.2	0.73	112	0	0	0	0	0	0	0	+	0
5	3	155	28.4	22.4	5.3	0.68	158	0	0	0	0	0	0	0	0	0
6	3	216	41.5	25.3	5.9	0.30	30	0	0	0	0	0	0	0	0	0
7	3	184	28.7	23.5	5.4	0.78	10	0	0	0	0	0	0	+	0	0
8	3	392	37.6	30	6.7	0.74	36	0	0	0	1	0	0	0	0	0
9	3	1193	52.3	44	9.8	0.83	116	0	0	0	3	0	0	0	0	0
10	3	1605	56.2	47.6	10.5	0.90	227	0	0	0	0	91	0	0	0	0
11	3	725	48.5	40	8.7	0.64	194	0	0	0	0	0	0	0	0	0
12	3	2390	67.2	55.5	12.2	0.79	874	0	0	0	0	93	0	0	0	0
1	1	423	34.5	27.6	7.5	1.03	66	0	0	0	0	0	0	0	0	0
2	1	454	35.3	29.3	7.4	1.03	5	0	0	0	0	1	0	0	0	0
3	1	296	32.7	26.5	6.3	0.85	36	0	0	0	0	0	0	0	0	0
4	1	216	30.4	25.3	5.4	0.77	37	0	0	0	0	0	0	0	0	0
5	1	267	30.9	25.3	6.5	0.90	126	0	0	0	0	0	0	0	0	0
6	1	305	33.8	27.5	6.6	0.79	162	0	0	0	0	1	0	0	0	0
7	1	144	27.4	22.4	4.8	0.70	71	0	0	1	0	0	0	+	0	0
8	1	108	25.5	20.8	4.2	0.65	18	0	0	0	0	0	0	0	0	0
9	1	372	35.1	29	6.3	0.86	14	0	0	1	0	0	0	0	0	0
10	1	359	35.1	28.9	6.6	0.83	220	0	1	0	0	0	0	0	0	0

N	S	W	TL	SL	BH	K	*P.l.*	*L.p.*	*A.a.*	*R.h.*	*P.t.*	*E.sp.*	*H.sp.*	*P.c.*	*D.s.*	*M.y.*
11	1	442	37.8	30.4	6.7	0.82	136	0	0	0	0	0	0	0	0	0
12	1	372	35.4	28.5	7.3	0.84	18	0	0	0	0	0	0	0	0	0
13	1	760	43.3	36	8.2	0.94	21	0	0	0	0	0	0	0	0	0
14	1	282	32.4	26.5	6.2	0.83	99	0	0	0	0	0	0	+	0	0
15	1	350	34	27.5	6.9	0.89	17	0	0	0	0	0	0	0	0	0
16	1	242	30.8	25	5.9	0.83	62	0	0	3	0	0	0	0	0	0
17	1	444	35.6	29.2	7.3	0.98	124	0	0	0	0	0	0	0	0	0
18	1	330	36.4	30.3	6.6	0.68	445	0	0	2	0	2	0	0	0	0
19	1	378	35	27.7	6.6	0.88	69	0	0	0	0	0	0	0	0	0
20	1	385	36.1	29.4	6.8	0.82	90	0	1	7	0	0	0	+	0	+
21	1	384	33.4	27.5	6.8	1.03	197	0	0	0	0	0	0	0	0	0
22	1	610	40.5	32.5	8.2	0.92	32	0	0	0	0	0	0	0	0	0
23	1	1742	56.3	47.2	11.2	0.98	363	0	0	0	0	7	0	0	0	0
24	1	3909	72	60.8	14	1.05	273	0	0	1	0	26	0	0	0	0
25	1	752	44.6	37.3	8.3	0.85	24	0	0	0	0	1	0	0	0	0
1	2	1685	54	46.6	11.7	1.07	223	0	0	0	0	7	0	+	0	0
2	2	1095	49.7	42	9.7	0.89	329	0	0	0	0	5	0	0	0	+
3	2	400	37.4	30.4	6.9	0.76	312	0	0	0	0	1	0	+	0	0
4	2	575	40.4	33.7	7.6	0.87	166	0	0	0	0	0	0	0	0	0
5	2	314	34.9	28.5	6.2	0.74	229	0	0	0	0	0	0	0	0	0
6	2	255	30.9	26.2	5.9	0.86	64	0	0	0	0	1	0	0	0	0
7	2	300	33.4	28.4	6.5	0.81	133	0	0	0	0	0	0	+	0	0
8	2	800	45.5	39.5	8.5	0.85	344	0	0	0	0	0	0	+	0	0
9	2	602	42.6	35.5	8.3	0.78	37	0	0	0	0	1	0	+	0	+
10	2	1315	51.3	42.6	10.6	0.97	91	0	0	0	0	4	0	0	+	0
11	2	335	35.6	29.2	5.6	0.74	243	0	0	0	0	0	0	0	0	0
12	2	2145	63.7	52.2	11.7	0.83	191	0	0	3	1	0	0	0	0	+
13	2	645	42.9	35.3	8.4	0.82	219	0	0	0	0	0	0	0	0	0
14	2	238	31.2	25.8	5.6	0.78	54	0	0	0	0	0	0	0	0	+
15	2	1563	54.8	45.6	11.2	0.95	90	0	0	0	0	0	0	+	0	0

N	S	W	TL	SL	BH	K	*P.l.*	*L.p.*	*A.a.*	*R.h.*	*P.t.*	*E.sp.*	*H.sp.*	*P.c.*	*D.s.*	*M.y.*
16	2	1521	55.4	45.6	10.3	0.89	401	0	0	0	0	24	0	0	0	0
17	2	1446	52.9	43.4	10.6	0.98	91	0	0	0	0	0	0	0	0	+
18	2	1640	56.8	47.7	11.7	0.89	216	0	0	0	0	2	0	0	0	0
19	2	2145	63.7	52.2	11.7	0.83	191	0	0	3	1	0	0	0	0	0
20	2	645	42.9	35.3	8.4	0.82	219	0	0	0	0	0	0	0	0	0
21	2	238	31.2	25.8	5.6	0.78	54	0	0	0	0	0	0	0	+	0
22	2	1563	54.8	45.6	11.2	0.95	90	0	0	0	0	0	0	0	0	0
23	2	1521	55.4	45.6	10.3	0.89	401	0	0	0	0	24	0	0	0	0
24	2	1446	52.9	43.4	10.6	0.98	91	0	0	0	0	0	0	0	0	0
1	3	490	40	33.3	6.6	0.77	95	0	0	0	0	0	0	0	0	0
2	3	415	37.8	31.1	7.2	0.77	71	0	0	0	0	0	0	0	0	0
3	3	640	36.9	29.7	6.9	1.27	53	0	0	0	0	0	0	0	0	0
4	3	355	36.3	29.8	6.3	0.74	33	0	0	0	0	3	0	0	0	0
5	3	260	31.9	26.3	6.3	0.80	1	0	0	0	0	0	0	0	+	0
6	3	400	35.6	29.8	6.7	0.89	59	0	0	0	0	0	0	0	0	0
7	3	435	37.2	30.8	6.6	0.85	41	0	0	0	0	0	0	0	+	0
8	3	270	31.4	26.4	5.9	0.87	4	0	0	0	0	0	0	0	0	0
9	3	405	37.6	30.6	6.7	0.76	74	0	0	0	0	0	0	0	0	0
10	3	600	40.4	33.2	7.5	0.91	92	0	0	0	0	0	0	0	0	0
11	3	295	34.6	28.2	6.3	0.71	54	0	0	11	0	0	0	0	0	0
12	3	475	39	31.4	7.7	0.80	6	0	0	0	0	0	0	0	0	0
13	3	260	31.4	25.8	6	0.84	1	0	0	1	0	0	0	0	0	0
14	3	305	33.5	28	5.9	0.81	14	0	0	0	0	0	0	0	0	0
15	3	230	30.1	25.1	5.5	0.84	86	0	0	0	0	0	0	0	0	0
16	3	200	30.4	25	5.7	0.71	79	0	0	0	0	0	0	0	0	0
17	3	370	35.7	29.8	6.4	0.81	95	0	0	0	0	1	0	0	0	+
18	3	375	36.6	30.4	6.7	0.76	29	0	0	0	0	0	0	0	0	0
19	3	155	28.2	23.3	4.5	0.69	89	0	0	0	0	0	0	0	0	+
20	3	355	34.5	29.2	6.7	0.86	9	0	0	0	0	0	0	0	0	0
21	3	610	36.5	30.5	6.6	1.25	22	0	0	0	0	0	0	0	0	0

N	S	W	TL	SL	BH	K	*P.l.*	*L.p.*	*A.a.*	*R.h.*	*P.t.*	*E.sp.*	*H.sp.*	*P.c.*	*D.s.*	*M.y.*
22	3	440	38.2	31	7.1	0.79	15	0	0	0	0	0	0	0	0	+
23	3	280	30.8	26	6.5	0.96	168	0	0	0	0	3	0	0	0	0
24	3	390	35.1	29.2	6.3	0.90	17	0	0	14	1	0	0	0	0	+
25	3	230	30.3	24.5	5.2	0.83	44	0	0	0	0	0	0	0	0	+
26	3	680	37.6	30.9	7.4	1.28	5	0	0	1	0	0	0	0	0	0
27	3	530	37.7	31.8	7.8	0.99	45	0	0	21	0	0	0	0	0	+
28	3	420	36.2	30.5	7.5	0.89	29	0	0	0	0	0	0	+	0	0
29	3	240	31.5	25.5	5.6	0.77	5	0	0	0	0	0	0	0	+	+
30	3	250	32	26.3	5.9	0.76	152	0	0	0	0	0	0	0	0	0
31	3	140	26.3	21.6	4.9	0.77	37	0	0	4	0	0	0	0	0	0
1	1	1000	47.6	39.8	8.9	0.93	149	0	0	7	0	0	0	+	0	0
2	1	1050	46.4	40	9.5	1.05	101	0	0	0	0	0	0	+	0	+
3	1	655	42.3	35.6	8	0.87	110	0	0	1	0	0	0	0	0	0
4	1	470	37.7	30.8	7.4	0.88	40	0	0	4	0	0	0	0	0	0
5	1	890	47.4	39.3	9.5	0.84	16	0	0	0	0	0	0	0	0	+
6	1	960	48.6	39.4	9.9	0.84	313	0	0	0	0	3	0	0	0	0
7	1	655	43	35.9	8.1	0.82	142	0	0	0	0	0	0	0	0	0
8	1	870	45.2	37.8	9.4	0.94	26	0	0	9	0	0	0	+	+	0
9	1	790	46.9	39	8.4	0.77	61	0	0	44	0	0	0	+	0	0
10	1	535	37.2	30.8	7.9	1.04	4	0	0	52	0	0	0	0	0	0
11	1	625	42.8	34.8	7.3	0.80	223	0	0	39	0	0	0	0	0	0
12	1	980	48.3	40.5	9.5	0.87	66	0	0	0	0	1	0	0	0	0
13	1	940	48.2	38.6	9.3	0.84	24	0	0	74	0	0	0	+	0	0
14	1	960	46.2	39.3	8.5	0.97	31	0	0	0	0	1	0	0	0	0
15	1	870	47.5	40.2	8.6	0.81	575	0	0	0	0	5	0	+	0	0
16	1	865	44.5	36.5	8.4	0.98	173	0	0	207	0	0	0	0	0	0
17	1	835	43.1	36	8.9	1.04	88	0	0	26	0	0	0	0	0	0
18	1	660	39.8	32.3	7.5	1.05	230	0	0	8	0	0	0	0	0	0
19	1	1000	48.4	40	8.9	0.88	98	0	0	0	0	0	0	+	0	0
20	1	635	41.4	34.5	7.6	0.89	121	0	0	0	0	5	0	0	0	0

N	S	W	TL	SL	BH	K	*P.l.*	*L.p.*	*A.a.*	*R.h.*	*P.t.*	*E.sp.*	*H.sp.*	*P.c.*	*D.s.*	*M.y.*
21	1	1035	49.3	40	9	0.86	175	0	0	0	0	2	0	+	0	+
22	1	745	44.9	36.8	7.5	0.82	293	0	0	56	0	0	0	0	0	0
23	1	1325	52	42.7	10.6	0.94	68	0	0	1	0	0	0	0	0	0
1	2	630	42.8	35	7.9	0.80	2	0	0	3	2	0	0	0	0	+
2	2	275	32.8	27.5	5.7	0.78	40	0	0	1	0	0	0	0	0	0
3	2	220	30.8	24.8	5	0.75	32	0	0	0	0	0	0	0	0	+
4	2	265	33	27.2	5.3	0.74	52	0	0	6	0	0	0	0	0	0
5	2	245	31.2	25.5	5.4	0.81	21	0	0	2	0	0	0	0	0	0
6	2	330	34.5	27.7	6	0.80	22	0	0	0	0	0	0	0	0	0
7	2	210	29.3	24.3	5.5	0.83	67	0	0	0	0	1	0	0	0	0
8	2	405	36	30	7.1	0.87	367	0	0	0	0	2	0	0	0	0
9	2	370	35.9	29.2	6.5	0.80	24	0	0	0	0	0	0	0	0	0
10	2	215	28.7	24.2	6	0.91	130	1	0	3	0	0	0	+	0	0
11	2	215	29.2	24	6.1	0.86	177	0	0	0	0	0	0	0	0	0
12	2	207	30	24.6	5.3	0.77	74	0	0	3	0	0	0	0	0	0
13	2	1000	47.7	40.4	9.6	0.92	84	0	0	0	0	0	0	+	0	0
14	2	835	45.2	37.8	8.3	0.90	63	0	0	1	0	0	0	+	+	0

Appendix I-B. Parasitological data of barbel collected from sampling site Kozloduy.

N	S	W	TL	SL	BH	K	*P.l.*	*L.p.*	*A.a.*	*R.h.*	*P.t.*	*E.sp.*	*H.sp.*	*P.c.*	*D.s.*	*M.y.*
1	2	153	25.7	21	4.5	0.90	49	0	0	2	0	0	0	0	0	0
2	2	396	35.3	28.5	6.8	0.90	182	0	0	2	0	0	0	0	0	0
3	2	252	34.1	28.3	4.8	0.64	28	0	0	1	0	0	0	0	0	+
4	2	256	31.1	24.6	5.3	0.85	20	0	0	27	0	0	0	0	0	0
5	2	460	39.5	32.9	6.4	0.75	41	0	0	51	0	0	0	0	+	0
6	2	315	33.6	26.9	6.6	0.83	124	0	0	1	0	1	0	0	0	0
7	2	227	31.3	25.9	4.9	0.74	23	0	0	3	2	0	0	0	0	0
8	2	151	26.4	21.8	4.7	0.82	101	0	0	0	0	0	0	0	0	+
9	2	531	39.3	32	7.8	0.87	55	0	0	9	0	0	0	0	0	0
10	2	540	39.3	31.9	7.1	0.89	121	0	0	0	0	0	0	0	0	+
11	2	202	30	24.4	5.2	0.75	40	0	0	0	0	0	0	0	0	0
12	2	297	32	27.1	6	0.91	32	0	0	5	0	0	0	0	0	0
13	2	2208	60.5	51.5	12	1.00	0	0	0	3	0	0	0	0	+	0
14	2	1336	51	41.2	10.5	1.01	4	0	0	2	0	0	0	0	0	0
1	3	390	33.8	28.7	7.3	1.01	43	0	0	1	0	0	0	0	0	0
2	3	375	34.6	28.6	7.2	0.91	53	0	0	8	0	0	0	0	0	0
3	3	525	41.4	34	7.3	0.74	40	0	0	0	0	0	0	+	0	0
4	3	390	34.9	28.5	7	0.92	27	0	0	6	0	0	0	+	0	0
5	3	373	35.8	29.2	6.8	0.81	46	0	0	12	1	0	0	0	+	0
6	3	437	37	29.8	7	0.86	59	0	0	12	0	0	0	0	0	0
7	3	586	40.3	33.6	7.7	0.90	44	0	0	1	0	2	0	0	0	0
8	3	418	36.7	30.2	7.2	0.85	128	0	0	0	1	0	0	0	0	0
9	3	624	41	33.5	8.1	0.91	148	0	0	0	0	0	0	+	0	+
10	3	360	33.7	28.8	6.9	0.94	174	0	0	0	0	0	0	0	0	0
11	3	283	32.5	26.3	6	0.82	207	0	0	0	0	0	0	0	0	0
12	3	561	40.1	33.2	7.8	0.87	32	0	0	6	0	0	0	0	0	0
13	3	549	42	34.1	7.4	0.74	115	0	0	0	0	3	0	0	0	+
14	3	410	35.5	30	7.2	0.92	85	0	0	6	0	0	0	+	0	0
15	3	375	34.1	27.6	7	0.95	107	0	0	0	1	0	0	0	0	0
16	3	195	29	24.2	5.2	0.80	41	0	0	2	0	0	0	0	0	0

N	S	W	TL	SL	BH	K	*P.l.*	*L.p.*	*A.a.*	*R.h.*	*P.t.*	*E.sp.*	*H.sp.*	*P.c.*	*D.s.*	*M.y.*
17	3	546	38	31	7.4	1.00	154	0	0	0	0	7	0	+	0	0
18	3	145	26.7	22.2	5.3	0.76	68	0	0	0	0	0	0	0	0	0
1	2	252	31.2	26.6	5.9	0.83	24	0	0	0	0	0	0	0	0	0
2	2	384	35.8	30.2	6.4	0.84	103	0	0	0	0	0	0	0	0	0
3	2	1309	52.9	44.4	10.4	0.88	69	0	0	0	0	0	0	0	0	0
4	2	966	48.4	40.8	9.5	0.85	168	0	0	0	0	1	0	0	0	0
5	2	1120	50.6	41.1	10	0.86	142	0	0	0	0	0	0	0	0	0
6	2	316	31.6	27.3	6.4	1.00	98	0	0	0	0	0	0	0	0	0
7	2	276	30.3	25.4	5.7	0.99	81	0	0	0	0	0	0	0	0	0
8	2	350	35.8	28.9	5.7	0.76	173	0	0	0	0	0	0	+	0	0
9	2	390	35.7	29.3	7.1	0.86	122	0	0	0	0	0	0	0	0	0
10	2	463	36.4	30.4	7.9	0.96	98	0	0	0	0	0	0	0	0	0
11	2	779	44.2	36.4	8.8	0.90	119	0	0	0	0	0	0	0	0	0
12	2	1070	49.9	41	9.6	0.86	252	0	0	0	0	26	0	0	0	0
13	2	270	31.8	26.4	5.8	0.84	128	0	0	0	0	0	0	+	0	+
14	2	483	36.5	31.5	7.4	0.99	45	0	0	0	0	0	0	0	0	0
15	2	480	39.3	31.7	6.7	0.79	53	0	0	0	0	0	0	0	0	0
16	2	580	40.6	32.8	8.2	0.87	44	0	0	0	0	0	0	0	0	0
17	2	536	38.4	32.8	7.3	0.95	170	0	0	0	0	0	0	0	0	+
18	2	1203	51.7	43.2	9.6	0.87	80	0	0	0	0	0	0	0	0	0
19	2	1310	53.4	44.2	10	0.86	222	0	0	1	0	0	0	0	0	0
20	2	1290	50.9	43.8	9.8	0.98	36	0	2	0	2	1	0	0	0	0
21	2	1135	50.9	42.8	9.4	0.86	138	0	0	0	0	0	0	0	0	0
22	2	1280	49.2	41.4	10.2	1.07	78	0	0	0	0	21	0	0	0	0
1	3	766	46.4	37.8	8.5	0.77	106	0	0	0	0	0	0	0	0	0
2	3	1155	52.6	44.8	9.4	0.79	31	0	0	0	0	4	0	0	0	0
3	3	965	49.5	40.4	9.3	0.80	63	0	0	0	0	68	0	0	0	0
4	3	1653	56.7	49	10.6	0.91	257	0	0	1	0	0	0	0	0	0
5	3	575	42.1	35.5	7.6	0.77	189	0	0	1	0	0	0	0	0	0
6	3	470	49.9	32.7	7.3	0.38	69	0	0	0	0	4	0	0	0	0
7	3	500	40.3	33.4	7.8	0.76	68	0	0	0	0	16	0	+	0	0
8	3	545	42	34.3	7.6	0.74	11	0	0	0	0	0	0	0	0	0
9	3	451	40	33.2	7.5	0.70	33	0	0	2	0	0	0	0	0	0

N	S	W	TL	SL	BH	K	*P.l.*	*L.p.*	*A.a.*	*R.h.*	*P.t.*	*E.sp.*	*H.sp.*	*P.c.*	*D.s.*	*M.y.*
10	3	362	37.1	30.4	6.9	0.71	8	0	0	5	0	0	0	0	+	0
11	3	581	41.6	33.5	7.9	0.81	150	0	0	0	0	11	0	0	0	0
12	3	215	31.7	25.6	5.7	0.67	61	0	0	0	0	0	0	0	0	0
13	3	1047	51.4	42.2	10.3	0.77	140	0	0	0	0	11	0	0	0	+
14	3	1785	50.2	49.9	11.9	1.41	237	0	0	0	0	31	0	0	0	0
15	3	1224	52.3	43.4	10.3	0.86	307	0	0	0	0	37	0	+	0	0
16	3	1350	54	44.6	10.9	0.86	33	0	0	2	0	1	0	0	0	0
17	3	1686	56.9	47.2	11.5	0.92	258	0	0	1	0	3	0	0	0	0
18	3	1270	55.2	46	10.2	0.76	60	0	0	1	0	0	0	0	0	0
19	3	1370	56.8	47	10.3	0.75	3	0	0	0	0	0	0	0	0	0
1	1	332	35	28.1	6.5	0.77	25	0	0	0	0	0	0	+	0	0
2	1	410	34	28.2	7.2	1.04	18	0	0	0	0	0	0	0	0	0
3	1	500	38.6	31.8	8	0.87	157	0	0	1	0	0	0	0	0	0
4	1	235	29	23.5	5.4	0.96	27	0	0	1	0	0	0	0	0	0
5	1	385	36.5	29.6	6.9	0.79	114	0	0	0	0	0	0	0	0	0
6	1	605	42	34.5	7.6	0.82	82	0	0	0	0	0	0	+	0	0
7	1	315	32.6	26.7	6.2	0.91	32	0	0	114	0	0	0	0	0	0
8	1	385	35.2	29	6.8	0.88	88	0	0	0	0	0	0	0	0	0
9	1	350	32.1	27	7.2	1.06	70	0	0	0	0	0	0	0	0	0
10	1	385	34.3	28.1	6.9	0.95	125	0	0	1	0	0	0	0	0	0
11	1	445	36.5	29.6	7.9	0.92	179	0	0	1	0	0	0	0	0	0
12	1	320	33	26.7	7	0.89	151	0	0	0	0	0	0	+	0	0
13	1	210	27.4	22.4	5.9	1.02	46	0	0	0	0	0	0	0	+	0
14	1	385	35	28.9	6.4	0.90	20	0	0	0	0	0	0	0	0	+
15	1	415	35.9	29.3	6.8	0.90	98	0	0	0	0	0	0	0	0	0
1	2	465	36.2	29.7	8.2	0.98	88	0	0	9	0	0	0	0	0	0
2	2	415	35.5	29.5	7.6	0.93	76	0	0	3	0	0	0	0	0	+
3	2	300	32	26.5	6.5	0.92	99	0	0	3	0	0	0	0	0	+
4	2	650	41.2	33.6	8.1	0.93	91	0	0	1	2	1	0	+	0	0
5	2	193	27.2	22.5	4.9	0.96	15	0	0	105	0	0	0	0	0	0
6	2	125	24.5	20	4.4	0.85	9	0	0	136	0	0	0	0	0	0
7	2	805	43.7	36	7.8	0.96	5	0	0	7	0	0	0	0	0	0
8	2	645	42.1	34.9	7.3	0.86	73	0	0	1	0	0	0	0	0	0

N	S	W	TL	SL	BH	K	*P.l.*	*L.p.*	*A.a.*	*R.h.*	*P.t.*	*E.sp.*	*H.sp.*	*P.c.*	*D.s.*	*M.y.*
9	2	710	40.3	33.4	8.5	1.08	197	0	0	1	0	0	0	+	0	0
10	2	255	29.7	24	5.6	0.97	74	0	0	26	0	0	0	0	0	0
11	2	270	30	24.5	5.2	1.00	55	0	0	132	0	0	0	0	0	+
12	2	1360	51.4	43.5	9.2	1.00	18	0	0	1	7	0	0	0	+	0
13	2	855	45.5	37.8	7.5	0.91	13	0	0	386	0	0	0	0	0	0
14	2	720	44.3	36.4	8.4	0.83	55	0	0	5	0	0	0	+	0	0
15	2	985	47.5	40.4	9.1	0.92	37	0	0	759	0	0	0	+	0	0
16	2	685	40.4	33.8	8.2	1.04	18	0	0	1	0	1	0	0	0	+
17	2	1000	45.7	39	8.8	1.05	9	0	0	21	0	0	0	+	0	0
18	2	470	35.5	29.2	7	1.05	4	0	0	0	0	0	0	+	0	+
19	2	475	37	31	7.7	0.94	94	0	0	13	0	0	0	+	0	0
20	2	440	35.8	29.5	7.2	0.96	18	0	0	2	2	0	0	0	0	0
21	2	465	37.2	30	7.5	0.90	10	0	0	196	0	0	0	0	0	0
22	2	195	27.4	22.1	5.5	0.95	69	0	0	2	0	0	0	0	+	0
23	2	580	40.2	33.5	8.3	0.89	174	0	0	1	0	0	0	+	0	+
24	2	375	34.1	27.6	7.2	0.95	112	0	0	30	0	0	0	0	0	0
25	2	440	36.6	29.5	7.4	0.90	46	0	0	7	0	0	0	0	+	0
26	2	395	32.7	28.3	7.6	1.13	109	0	0	5	0	0	0	0	0	0
27	2	300	42.6	27.4	6.8	0.39	63	0	0	1	0	0	0	0	0	+
28	2	330	32.7	26.8	6.9	0.94	60	0	0	2	0	0	0	0	0	0
29	2	625	40.3	32.8	9	0.95	235	0	0	1	0	0	0	0	0	0
30	2	410	37.6	31.5	6.4	0.77	9	0	0	0	0	0	0	+	+	+
31	2	375	35.5	29	6.9	0.84	127	0	0	10	0	1	0	0	0	0
32	2	360	34.3	27.8	6.9	0.89	69	0	0	9	0	0	0	0	+	0
33	2	565	40.1	33.5	8.2	0.88	38	0	0	0	0	8	0	+	0	0
34	2	570	39.8	32	7.6	0.90	63	0	0	27	0	0	0	0	0	0
35	2	385	33.3	27.8	6.6	1.04	6	0	0	0	0	0	0	0	0	0
36	2	240	29.1	23.8	5.9	0.97	77	0	0	4	0	0	0	0	0	0
1	3	380	35.1	28.5	6.6	0.88	28	0	0	0	0	0	0	0	0	0
2	3	405	35.4	29.8	6.6	0.91	43	0	0	0	0	0	0	0	0	0
3	3	280	31.4	26	5.7	0.90	60	0	0	6	0	0	0	0	+	0
4	3	320	33	27.4	6.3	0.89	68	0	0	7	0	0	0	0	0	+
5	3	455	35	30.5	6.9	1.06	120	0	0	0	0	0	0	0	0	0

N	S	W	TL	SL	BH	K	*P.l.*	*L.p.*	*A.a.*	*R.h.*	*P.t.*	*E.sp.*	*H.sp.*	*P.c.*	*D.s.*	*M.y.*
6	3	520	40.5	33.2	7.4	0.78	383	0	0	1	0	0	0	0	0	0
7	3	465	49.2	33	7.2	0.39	89	0	0	0	0	0	0	0	0	0
8	3	450	37	31	7.5	0.89	158	0	0	0	0	0	0	0	0	0
9	3	180	27.8	22.5	5.2	0.84	6	0	0	1	0	0	0	0	0	0
10	3	345	36.3	29.8	6.4	0.72	2	0	0	11	0	0	0	0	0	0
11	3	325	33.8	27.8	6.8	0.84	66	0	0	0	0	0	0	0	0	0
12	3	470	38.1	31.7	7.4	0.85	72	0	0	0	0	0	0	0	0	+
13	3	455	37	29.8	7	0.90	24	0	0	0	0	0	0	0	0	+
14	3	180	29	23.8	5.4	0.74	0	0	0	0	0	0	0	0	0	0
15	3	455	38.5	32	7.6	0.80	90	0	0	0	0	0	0	0	0	+
16	3	210	30.1	25.2	5.5	0.77	5	0	0	0	0	0	0	0	0	0
17	3	140	26.5	22	4.4	0.75	2	0	0	1	0	0	0	0	+	+
18	3	275	32.3	26.4	6.2	0.82	22	0	0	1	0	0	0	+	0	0
19	3	400	34	28.4	6.9	1.02	14	0	0	0	0	0	0	0	0	0
20	3	275	31.3	25.7	6.4	0.90	11	0	0	0	0	0	0	0	0	0
21	3	595	39	32.9	8.2	1.00	3	0	0	0	0	0	0	0	0	+
22	3	210	31.7	25.8	5.1	0.66	249	0	0	1	0	0	0	0	0	0
23	3	300	32.4	27.4	6.1	0.88	5	0	0	0	0	0	0	0	0	0
24	3	295	32.7	26.4	6.1	0.84	119	0	0	0	0	0	0	+	0	0
25	3	625	41.3	33.8	8.5	0.89	74	0	0	6	0	0	0	0	0	0
26	3	625	43.6	36.7	7.7	0.75	47	0	0	0	0	1	0	+	+	+
27	3	355	31.1	26.6	6.7	1.18	51	0	0	2	0	0	0	0	0	0
28	3	290	31.2	25.9	6.6	0.95	65	0	0	0	0	0	0	0	0	0
29	3	250	30.2	25	6	0.91	16	0	0	1	0	0	0	0	0	0
30	3	455	36.9	30.5	7.7	0.91	27	0	0	0	0	0	0	0	0	0
31	3	215	26.2	21.6	6.3	1.20	85	0	0	1	0	0	0	0	0	0
32	3	680	35.7	30.1	7.6	1.49	123	0	0	0	0	0	0	0	0	0
33	3	515	38.8	33	6.7	0.88	403	0	0	0	0	0	0	+	0	0
34	3	375	34.9	28.7	6.4	0.88	117	0	0	0	0	0	0	0	0	0
1	1	870	45.4	37	8.7	0.93	14	0	0	0	0	0	0	0	0	0
2	1	1090	49.6	40.7	10.3	0.89	384	0	0	0	0	9	0	0	0	0
3	1	685	42.5	35.3	8.3	0.89	176	0	0	138	0	0	0	0	0	0
4	1	865	46	36.9	9.4	0.89	35	0	0	0	0	0	0	+	0	+

N	S	W	TL	SL	BH	K	*P.l.*	*L.p.*	*A.a.*	*R.h.*	*P.t.*	*E.sp.*	*H.sp.*	*P.c.*	*D.s.*	*M.y.*
5	1	835	47.2	39.3	9.6	0.79	86	0	0	0	0	0	0	0	0	0
6	1	740	43.6	36.5	8.6	0.89	31	0	0	0	0	0	0	0	0	0
7	1	1050	45	39	9.8	1.15	133	0	0	0	0	11	0	0	+	0
8	1	1000	46	36.9	8.3	1.03	21	0	0	189	0	0	0	+	0	0
9	1	965	47.1	38.5	9.8	0.92	37	0	0	0	0	1	0	0	0	0
10	1	1105	52.3	42.5	9.8	0.77	153	0	0	203	0	0	0	0	0	0
11	1	925	46.4	39.5	8.8	0.93	124	0	0	2	0	10	0	0	0	0
12	1	120	26	21.2	4.4	0.68	5	0	0	6	0	0	1	0	0	0
13	1	220	30.5	24.6	5.7	0.78	69	0	0	1	0	0	0	0	0	0
14	1	590	42.6	35.5	7.1	0.76	220	0	0	1	0	2	0	0	0	0
15	1	630	42	34.9	7.4	0.85	45	0	0	0	0	2	0	0	0	0
16	1	1125	48.9	40.9	10	0.96	134	0	0	87	0	0	0	0	0	0
17	1	505	37.3	31.4	7.8	0.97	17	0	0	0	0	0	0	0	0	0
18	1	455	38	31.4	7.3	0.83	148	0	0	1	0	0	0	0	0	0
19	1	395	35.6	29.3	6.4	0.88	15	0	0	0	0	0	0	0	0	+
20	1	545	37.6	31.3	7.5	1.03	52	0	0	253	0	0	0	0	0	0
21	1	885	47.8	39.6	8.4	0.81	29	0	0	1	0	1	0	0	+	+
22	1	530	41.3	33.7	7.5	0.75	67	0	0	0	0	0	0	+	0	0
23	1	460	38.5	33.5	7	0.81	20	0	0	0	0	0	0	+	0	0
24	1	580	38.5	31.4	7.6	1.02	17	0	0	0	0	0	0	+	0	0
1	2	590	40.9	33.5	8	0.86	93	0	0	0	0	1	0	0	0	0
2	2	430	36	29.1	7.2	0.92	12	0	0	0	0	0	0	0	0	0
3	2	820	48.9	40.1	8.2	0.70	6	0	0	0	0	2	0	+	0	+
4	2	955	48	38.5	8.5	0.86	48	0	0	1	0	0	0	0	0	0
5	2	1025	48.3	40.4	9.8	0.91	19	0	0	1	0	0	0	0	+	+
6	2	320	36	29.7	5.1	0.69	29	0	0	1	0	1	0	0	0	0
7	2	400	37.5	31.1	6	0.76	41	0	0	0	0	0	0	0	+	0
8	2	495	38.2	31.4	7.3	0.89	59	0	0	0	0	0	0	0	0	0
9	2	800	48.4	40.2	8.5	0.71	137	0	0	2	0	0	0	0	0	0
10	2	845	46	38.4	8	0.87	424	0	0	0	0	0	0	+	0	0
11	2	755	45.7	37.3	8.2	0.79	87	0	0	0	0	0	0	0	0	+

Appendix I-C. Parasitological data of barbel collected from sampling site Silistra.

N	S	W	TL	SL	BH	K	*P.l.*	*L.p.*	*A.a.*	*R.h.*	*P.t.*	*E.sp.*	*H.sp.*	*P.c.*	*D.s.*	*M.y.*
1	2	430	34.1	29	7.5	1.08	77	0	0	5	0	0	0	0	0	0
2	2	410	34.6	29	6.6	0.99	112	0	0	1	0	0	0	+	0	0
3	2	660	38.5	32.4	8.6	1.16	60	0	0	0	0	0	0	0	0	0
4	2	670	40.8	34.1	9.3	0.99	126	0	0	0	0	0	0	+	0	0
5	2	640	37.5	31.2	9.2	1.21	131	0	0	1	2	0	0	+	0	0
6	2	535	38.2	32.6	8.1	0.96	128	0	0	0	0	0	0	0	0	0
7	2	670	39.5	33.2	9	1.09	42	0	0	1	1	0	0	+	0	+
8	2	610	39.5	32.1	8.9	0.99	208	0	0	2	0	0	0	+	0	0
9	2	550	39.6	32.8	8.2	0.89	167	0	0	26	0	0	0	+	0	0
10	2	1640	50	41.6	12	1.31	82	0	0	0	0	0	0	0	0	0
11	2	1395	52.5	44	11.1	0.96	236	0	0	0	0	0	0	+	+	0
12	2	1065	48.3	39.5	10.9	0.95	282	0	0	0	1	0	0	+	0	0
13	2	1785	54.8	44.5	12.5	1.08	81	0	0	1	0	6	0	0	0	0
14	2	790	41.8	35	7.5	1.08	523	0	0	0	0	1	0	0	0	0
1	3	739	47	38	9	0.71	114	0	0	3	0	1	0	0	0	0
2	3	600	39.4	32.6	8.2	0.98	22	0	0	1	0	0	0	0	0	0
3	3	420	34.3	28.5	7.4	1.04	57	0	0	0	0	0	0	0	0	0
4	3	420	34.3	28.2	6.8	1.04	94	0	0	26	0	0	0	0	+	0
5	3	530	37.8	31.3	7.8	0.98	86	0	0	46	0	0	0	0	0	0
6	3	555	38.4	32.2	8.5	0.98	88	0	0	32	0	0	0	0	0	0
7	3	900	45	37.7	10	0.99	120	0	0	0	0	0	0	+	0	0
8	3	890	43.8	37	8	1.06	166	0	0	0	0	1	0	0	0	0
9	3	770	45.2	36.7	9.1	0.83	282	0	0	0	0	0	0	+	0	0
10	3	655	38.4	32.3	8.7	1.16	24	0	0	39	0	0	0	+	0	0
11	3	475	36.6	29.9	7.5	0.97	4	0	0	12	0	0	0	0	0	0
12	3	530	36.5	30	7.9	1.09	4	0	0	51	0	0	0	0	0	0
1	1	400	34.8	28.5	7.3	0.95	0	0	0	0	0	0	0	0	0	0

N	S	W	TL	SL	BH	K	*P.l.*	*L.p.*	*A.a.*	*R.h.*	*P.t.*	*E.sp.*	*H.sp.*	*P.c.*	*D.s.*	*M.y.*
2	1	735	41	34.5	8.4	1.07	263	0	0	0	0	0	0	+	0	0
3	1	745	38.8	31.7	9.4	1.28	195	0	0	0	0	0	0	+	0	0
4	1	485	36.5	30	7.9	1.00	37	0	0	0	0	0	0	+	0	0
5	1	815	45.1	37.5	9	0.89	104	0	0	66	0	0	0	+	0	0
6	1	1050	49	40.3	10	0.89	114	0	0	0	1	1	0	+	0	+
7	1	965	50	40	10	0.77	68	0	0	27	0	0	0	0	+	0
8	1	885	47.2	38	9.1	0.84	13	0	0	2	0	0	0	0	0	0
9	1	995	44.9	36.9	9.1	1.10	164	0	0	0	0	0	0	0	0	0
10	1	930	44.2	36.1	9.8	1.08	10	0	0	0	0	0	0	0	0	0
1	2	1350	53.1	44.2	9.3	0.90	5	0	0	761	0	0	0	0	0	0
2	2	1385	52.3	42.3	10.5	0.97	287	0	0	1	5	3	0	0	0	0
3	2	915	48.5	40.4	8.4	0.80	99	0	0	72	0	0	0	0	0	+
4	2	875	44.6	36.5	9.1	0.99	39	0	0	0	0	0	0	0	0	0
5	2	1070	51	42.8	9.3	0.81	81	0	0	0	0	0	0	0	0	0
6	2	1175	48.2	39.5	9	1.05	31	0	0	0	0	0	0	0	0	0
7	2	995	47.2	38.5	8.5	0.95	45	0	0	492	0	0	0	+	0	0
8	2	1265	47.5	39.8	11.7	1.18	409	0	0	0	0	2	0	0	0	+
9	2	1105	45.4	37	11.1	1.18	80	0	0	0	0	0	0	+	0	0
10	2	920	47.1	38.7	9.5	0.88	66	0	0	0	0	0	0	0	0	0
11	2	560	40.4	33	8.1	0.85	23	0	0	8	0	0	0	0	0	0
12	2	965	45.3	37.3	9.3	1.04	181	0	0	0	0	0	0	+	0	0
13	2	1165	47.9	39.7	10.8	1.06	19	0	0	0	0	0	0	0	0	+

Appendix II: Element concentrations in barbel tissues and *P. laevis* used in **Chapter 2**.

Used abbreviations in the appendix:

N: barbel number

S: Sample tissue (M – muscle; I – intestine; L – liver; *P.l. – Pomphorhynchus laevis*)

Appendix II-A. Element concentrations in host tissues and *P. laevis* of heavily infected barbels.

N	S	As	Cd	Co	Cu	Fe	Mn	Mo	Ni	Pb	Sn	V	Zn
1	M	0.123	0.015	0.009	0.731	13.134	0.265	0.006	0.229	0.007	0.002	0.045	4.515
2	M	0.238	0.176	0.025	0.964	15.961	0.804	0.009	1.808	0.027	0.005	0.046	4.941
3	M	0.095	0.016	0.011	1.235	8.269	0.313	0.005	0.163	0.000	0.003	0.032	4.069
4	M	0.085	0.007	0.027	1.297	8.693	0.230	0.007	0.335	0.016	0.004	0.035	3.352
5	M	0.043	0.028	0.018	0.691	13.831	0.329	0.008	0.506	0.021	0.004	0.050	4.069
6	M	0.140	0.006	0.007	1.226	9.347	0.259	0.007	1.188	0.006	0.003	0.032	3.986
7	M	0.180	0.011	0.011	1.217	5.372	0.347	0.005	0.542	n.d.	0.000	0.025	3.427
8	M	0.406	0.016	0.013	0.428	7.910	0.282	0.004	0.249	0.003	0.003	0.025	2.369
9	M	0.181	0.012	0.009	1.238	6.389	0.266	0.003	0.221	0.047	0.004	0.021	3.498
1	I	0.425	0.060	0.030	1.843	27.648	1.369	0.023	0.806	0.040	0.008	0.083	11.199
2	I	0.469	0.161	0.052	3.816	37.593	2.975	0.041	0.290	0.039	0.008	0.104	10.227
3	I	0.160	0.058	0.074	1.678	68.852	13.054	0.025	1.370	0.421	0.008	0.237	7.558
4	I	0.301	0.085	0.088	5.993	69.514	4.250	0.030	1.311	0.126	0.015	0.201	10.393
5	I	0.217	0.126	0.190	6.918	143.787	11.371	0.042	1.288	0.303	0.017	0.439	21.279
6	I	0.422	0.050	0.049	4.318	46.778	2.991	0.046	1.536	0.019	0.010	0.201	12.703
7	I	0.496	0.127	0.239	6.390	158.238	18.169	0.018	2.065	0.482	0.009	0.783	15.106
8	I	*	0.175	0.142	2.222	90.310	5.119	0.109	0.756	0.199	0.037	0.244	14.086
9	I	0.495	0.098	0.173	3.733	120.122	9.070	0.018	1.237	0.228	0.009	0.394	8.823
1	L	0.400	0.160	0.028	5.837	87.927	0.923	0.087	0.043	0.027	0.014	0.375	19.413
2	L	0.354	0.187	0.035	9.739	82.479	1.122	0.117	0.383	0.133	0.035	0.272	22.913
3	L	0.229	0.085	0.045	7.243	60.049	1.168	0.101	0.375	0.038	0.010	0.179	17.080
4	L	0.245	0.204	0.033	178.782	83.719	0.753	0.243	0.404	0.034	0.036	0.267	32.103
5	L	0.135	0.181	0.129	5.597	762.201	3.352	0.088	0.425	1.104	0.016	0.331	16.005
6	L	0.343	0.101	0.032	8.677	36.935	1.046	0.231	0.267	0.032	0.043	0.203	16.826
7	L	0.616	0.111	0.038	25.398	44.419	1.079	0.142	0.484	0.016	0.005	0.075	20.777
8	L	*	0.232	0.037	10.823	129.575	1.782	0.124	1.550	0.035	0.006	0.295	25.277
9	L	0.557	0.118	0.033	10.654	53.948	1.267	0.092	0.058	0.000	0.007	0.125	18.855
1	P.l.	1.731	1.599	0.069	72.826	36.385	3.617	0.182	0.272	7.708	n.d.	0.125	34.287
2	P.l.	1.732	2.616	0.074	91.379	63.844	4.382	0.059	0.190	10.348	n.d.	0.277	128.232
3	P.l.	2.156	2.275	0.102	90.997	44.627	4.490	0.048	0.251	4.959	n.d.	0.194	178.334
4	P.l.	1.505	3.442	0.096	159.436	45.643	4.656	0.035	0.300	9.222	n.d.	0.162	162.001
5	P.l.	2.436	3.583	0.148	130.552	75.201	8.671	0.072	0.552	51.803	n.d.	0.255	208.329
6	P.l.	0.432	1.523	0.056	73.184	24.229	4.783	0.022	0.234	6.861	n.d.	0.118	40.123
7	P.l.	1.594	2.908	0.138	99.337	42.942	5.210	0.028	0.387	11.747	n.d.	0.167	65.568
8	P.l.	*	0.915	0.107	54.935	152.905	28.407	0.014	0.648	3.491	n.d.	0.317	61.954
9	P.l.	1.261	1.715	0.099	63.732	49.728	5.043	0.015	0.435	5.692	n.d.	0.173	70.784

n.d.: concentrations below detection limit

*: values not taken

Appendix III-B. Element concentrations in host tissues and *P. laevis* of slightly infected barbells.

N	S	As	Cd	Co	Cu	Fe	Mn	Mo	Ni	Pb	Sn	V	Zn
1	M	0.035	0.026	0.011	0.960	13.786	0.545	0.007	1.364	0.011	0.003	0.029	3.645
2	M	0.218	0.025	0.023	1.104	15.791	0.324	0.008	0.193	n.d.	0.003	0.055	4.003
3	M	0.059	0.022	0.013	0.904	9.656	0.463	0.007	0.337	0.007	0.001	0.026	3.674
4	M	0.049	0.013	0.010	1.071	10.857	0.739	0.008	0.506	0.038	0.004	0.029	3.969
5	M	0.116	0.002	0.012	0.610	5.814	0.207	0.000	0.272	0.002	0.002	0.020	2.846
6	M	0.349	0.032	0.008	1.171	4.023	0.202	0.008	0.075	0.009	0.007	0.037	4.721
7	M	0.060	0.005	0.008	0.243	4.655	0.368	0.004	0.128	n.d.	0.000	0.022	2.915
8	M	0.058	0.018	0.004	1.233	7.897	0.276	0.009	0.077	0.002	0.002	0.032	3.128
9	M	0.382	0.078	0.016	2.797	6.202	0.280	0.008	1.798	0.003	0.002	0.034	3.681
1	I	0.166	0.257	0.063	4.482	54.507	3.183	0.045	1.252	0.174	0.008	0.104	11.295
2	I	0.521	0.217	0.077	9.775	55.435	3.451	0.044	1.256	0.139	0.014	0.178	11.123
3	I	0.190	0.212	0.042	4.417	26.020	3.414	0.053	0.734	0.131	0.006	0.073	11.830
4	I	0.211	0.246	0.107	7.013	77.421	6.377	0.038	2.288	0.284	0.010	0.225	13.525
5	I	0.144	0.019	0.013	1.850	9.965	0.846	0.029	0.856	0.067	0.007	0.054	14.772
6	I	0.622	0.450	0.187	11.781	128.525	15.224	0.100	1.493	0.366	0.035	0.553	14.465
7	I	0.332	0.169	0.196	1.944	111.115	18.227	0.036	1.661	0.241	0.009	0.292	9.217
8	I	0.070	0.435	0.037	4.323	29.648	1.552	0.053	0.387	0.023	0.003	0.041	14.737
9	I	0.817	0.259	0.168	17.227	132.828	13.448	0.029	1.698	0.355	0.007	0.519	12.980
1	L	0.177	0.149	0.032	8.870	101.215	1.902	0.134	0.532	0.098	0.012	0.093	16.971
2	L	0.786	0.188	0.055	6.431	61.371	1.714	0.086	0.836	0.045	0.007	0.233	14.733
3	L	0.345	0.304	0.039	30.341	92.301	2.456	0.205	0.394	0.046	0.013	0.113	27.322
4	L	0.195	0.137	0.047	15.912	119.598	4.492	0.105	0.456	0.065	0.004	0.090	21.549
5	L	0.839	0.052	0.024	10.107	84.958	1.114	0.093	0.117	0.006	0.013	0.290	27.191
6	L	0.963	0.136	0.041	5.500	20.414	1.738	0.100	0.383	0.005	n.d.	0.064	13.168
7	L	0.173	0.157	0.033	4.574	67.664	1.528	0.101	0.064	0.000	0.001	0.158	12.784
8	L	0.189	0.195	0.030	17.607	88.017	1.390	0.150	6.150	0.033	0.008	0.131	20.835
9	L	1.239	0.092	0.033	15.374	39.185	0.983	0.081	3.719	0.016	0.007	0.121	17.329
1	P.l.	0.985	3.794	0.161	112.572	49.427	17.030	0.291	0.597	19.753	n.d.	0.086	589.719
2	P.l.	1.263	5.257	0.133	387.022	65.487	3.238	0.061	0.337	9.978	n.d.	0.124	46.968
3	P.l.	1.940	13.127	0.089	137.278	43.133	17.327	0.065	0.627	16.308	n.d.	0.078	360.795
4	P.l.	0.387	5.316	0.089	111.940	30.769	18.891	n.d.	0.382	11.515	n.d.	0.088	457.576
5	P.l.	0.304	0.528	0.025	20.667	15.820	2.532	n.d.	0.000	2.267	n.d.	0.050	20.470
6	P.l.	1.958	14.666	0.076	111.687	26.227	4.741	0.035	0.344	15.109	n.d.	0.126	228.797
7	P.l.	*	*	*	*	*	*	*	*	*	*	*	*
8	P.l.	0.253	3.891	0.062	42.600	28.013	7.505	0.009	0.418	5.307	n.d.	0.052	43.652
9	P.l.	0.470	0.990	0.089	57.236	20.107	4.305	0.004	0.286	1.438	n.d.	0.121	36.901

n.d.: concentrations below detection limit
*: values not taken

Appendix II-C. Element concentrations in host tissues and females and males of *P. laevis*.

N	S	As	Cd	Co	Cu	Fe	Mn	Mo	Ni	Pb	Sn	V	Zn
1	M	0.050	0.007	0.004	0.587	6.139	0.284	0.004	0.112	0.014	0.003	0.011	2.822
2	M	0.196	0.012	0.018	0.856	7.354	0.495	0.005	0.549	0.003	0.001	0.017	3.376
3	M	0.204	0.026	0.009	1.219	9.504	0.246	0.005	0.342	0.000	0.004	0.038	3.791
4	M	0.300	0.025	0.010	1.186	11.635	0.272	0.006	0.227	0.017	0.004	0.043	4.039
5	M	0.128	0.015	0.016	0.595	6.737	0.283	0.005	0.148	0.006	0.004	0.031	4.598
6	M	0.110	0.043	0.035	1.193	13.374	1.455	0.008	2.588	0.016	0.001	0.039	3.302
7	M	0.160	0.042	0.023	1.715	17.603	0.342	0.029	0.325	0.026	0.004	0.037	4.526
8	M	0.342	0.010	0.010	0.674	6.326	0.192	0.004	0.466	0.017	0.002	0.024	2.844
1	I	0.140	0.062	0.035	1.579	22.278	2.923	0.089	0.462	0.024	0.006	0.044	8.126
2	I	0.667	0.217	0.414	8.076	283.876	33.500	0.043	2.096	1.379	0.018	1.033	10.602
3	I	0.390	0.099	0.070	2.883	38.581	2.173	0.023	2.540	0.194	0.010	0.118	10.740
4	I	0.509	0.127	0.053	7.520	36.942	2.587	0.027	0.804	0.107	0.008	0.105	8.210
5	I	0.461	0.115	0.144	5.542	112.767	8.623	0.021	1.027	0.266	0.011	0.425	9.483
6	I	0.558	0.198	0.452	9.707	196.072	39.889	0.039	2.507	1.282	0.010	1.004	11.575
7	I	0.290	0.131	0.154	5.781	143.294	12.036	0.019	1.081	0.292	0.002	0.719	8.794
8	I	0.608	0.198	0.176	8.721	111.702	9.010	0.019	2.322	0.335	0.003	0.405	13.584
1	L	0.488	0.171	0.032	16.792	116.333	2.207	0.161	0.396	0.097	0.015	0.052	21.816
2	L	0.729	0.100	0.072	9.838	51.846	2.117	0.108	0.214	0.066	0.024	0.055	16.441
3	L	0.394	0.293	0.037	18.403	80.057	0.911	0.101	0.113	0.086	0.016	0.144	22.953
4	L	0.805	0.090	0.038	9.739	47.926	1.099	0.209	0.201	0.143	0.061	0.186	16.146
5	L	0.303	0.093	0.026	4.565	76.498	0.885	0.105	0.277	0.003	0.016	0.227	16.205
6	L	0.471	0.320	0.110	20.237	95.855	2.821	0.243	0.326	0.073	0.008	0.300	22.998
7	L	0.440	0.105	0.034	6.196	45.563	0.759	0.077	0.589	0.019	0.004	0.084	13.455
8	L	0.880	0.079	0.020	8.285	29.439	0.826	0.062	0.159	0.010	0.004	0.049	13.989
1	*P.l.* ♂	0.284	1.266	0.102	25.046	34.251	10.164	0.392	0.559	3.335	0.143	0.054	51.338
2	*P.l.* ♂	0.820	2.030	0.157	87.794	43.857	14.928	0.058	1.167	10.919	0.020	0.141	73.472
3	*P.l.* ♂	2.232	2.381	0.074	50.133	36.770	4.588	0.019	0.466	10.273	0.005	0.102	33.353
4	*P.l.* ♂	2.988	3.551	0.077	64.596	44.711	5.567	0.034	0.408	10.865	n.d.	0.118	89.820
5	*P.l.* ♂	3.861	1.615	0.083	51.662	33.999	4.117	0.023	1.690	3.863	0.010	0.097	36.158
6	*P.l.* ♂	1.496	2.867	0.295	80.038	54.780	24.787	0.031	1.149	16.145	0.005	0.225	43.792
7	*P.l.* ♂	2.038	2.437	0.094	40.771	32.482	3.801	0.017	0.361	6.402	n.d.	0.122	49.566
8	*P.l.* ♂	0.752	2.931	0.079	130.318	41.992	3.819	0.013	0.915	15.278	0.000	0.121	69.439
1	*P.l.* ♀	0.406	2.573	0.090	43.225	25.163	9.768	0.197	0.314	5.657	n.d.	0.061	152.305
2	*P.l.* ♀	0.949	2.580	0.221	122.360	54.722	17.824	0.053	0.805	8.956	n.d.	0.254	154.508
3	*P.l.* ♀	2.836	2.178	0.078	47.181	33.968	4.907	0.015	0.536	8.960	n.d.	0.108	65.014
4	*P.l.* ♀	2.240	3.538	0.080	92.528	33.372	3.785	0.022	0.367	11.698	n.d.	0.121	70.738
5	*P.l.* ♀	4.034	1.659	0.089	42.370	33.810	4.224	0.017	0.412	2.997	n.d.	0.145	53.999
6	*P.l.* ♀	1.463	2.517	0.281	87.445	52.640	21.294	0.035	1.108	15.544	n.d.	0.258	51.264
7	*P.l.* ♀	2.601	3.189	0.104	53.952	33.844	5.084	0.021	0.503	8.120	n.d.	0.139	63.857
8	*P.l.* ♀	0.820	2.772	0.089	130.160	33.196	4.621	0.016	0.634	16.576	n.d.	0.162	116.758

n.d.: concentrations below detection limit

Appendix III: Water temperature data (in °C)at two localities of Danube River for the period 2005-2006 (data from TNMN (2009)).

Month	Novo Selo (5 km upstream of Vidin)		Iskar (40 km downstream of Kozloduy)	
	2005	2006	2005	2006
J	4.9	2.8	8.8	6.9
F	1.5	6.4	6.5	6.2
M	2.1	5.3	3	5.3
A	11.5	10.6	10.4	11.4
M	16.2	15.4	15.6	18
J	17.5	16.8	21.4	20
J	23.9	24.9	24.2	25.1
A	24.2	25.1	27.6	*
S	15.2	21.1	22.4	24
O	15.5	18.3	18.8	22.9
N	10.6	11.2	11.2	14.7
D	7.3	7.2	8.3	11

* value is not available

Appendix IV. Morphological data of *Pomphorhynchus laevis* used in **Chapter 3**.

In the Appendix were used the following abbreviations:

N: Fish number

S: Season (1 – spring, 2 – summer, 3 – autumn)

W: Weight of *P. laevis* infracomunity (g)

N *P.l.*: Number of individuals

MAW: Mean acanthocephalan weigh (g)

N	S	W	N *P.l.*	MAW
1	1	0.375	26	0.014
2	1	0.421	18	0.023
3	1	2.382	157	0.015
4	1	2.124	114	0.019
5	1	1.025	82	0.013
6	1	1.852	88	0.021
7	1	1.832	125	0.015
8	1	3.784	179	0.021
1	2	1.471	88	0.017
2	2	1.376	76	0.018
3	2	1.868	99	0.019
4	2	1.225	91	0.013
5	2	0.827	55	0.015
6	2	1.213	55	0.022
7	2	1.409	37	0.038
8	2	3.945	109	0.036
1	3	0.441	61	0.007
2	3	1.105	68	0.016
3	3	0.720	89	0.008
4	3	1.247	66	0.019
5	3	1.934	72	0.027
6	3	0.846	74	0.011
7	3	0.641	65	0.010
8	3	1.280	85	0.015

Lebenslauf Milen Nachev

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1997-2002	Studium an der Universität Sofia (Fakultät für Biologie) Fach: Ökologie und Umweltschutz; Magister-Abschluss
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Unterschrift**:**

/Dipl.-Ökol. Milen Nachev/

Erklärung:

Hiermit erkläre ich, gem. § 6 Abs. 2, Nr. 7 der Promotionsordnung der Math.-Nat.-Fachbreiche zur Erlangung der Dr. rer. nat., dass ich das Arbeitgebiet, dem das Thema „**Bioindication capacity of fish parasites for the assessment of water quality in the Danube River**“ zuzuordnen ist, in Forschung und Lehre vertrete und den Antrag von **Milen Nachev** befürworte.

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Erklärung:

Hiermit erkläre ich, gem. § 6 Abs. 2, Nr. 6 der Promotionsordnung der Math.-Nat.-Fachbereiche zur Erlangung des Dr. rer. nat., dass ich die vorliegende Dissertation selbständig verfasst und mich keiner anderen als der angegebenen Hilfsmittel bedient habe.

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Erklärung:

Hiermit erkläre ich, gem. § 6 Abs. 2, Nr. 8 der Promotionsordnung der Math.-Nat.-Fachbereiche zur Erlangung des Dr. rer. nat., dass ich keine anderen Promotionen bzw. Promotionsversuche in der Vergangenheit durchgeführt habe und dass diese Arbeit von keiner anderen Fakultät/Fachbereich abgelehnt worden ist.

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